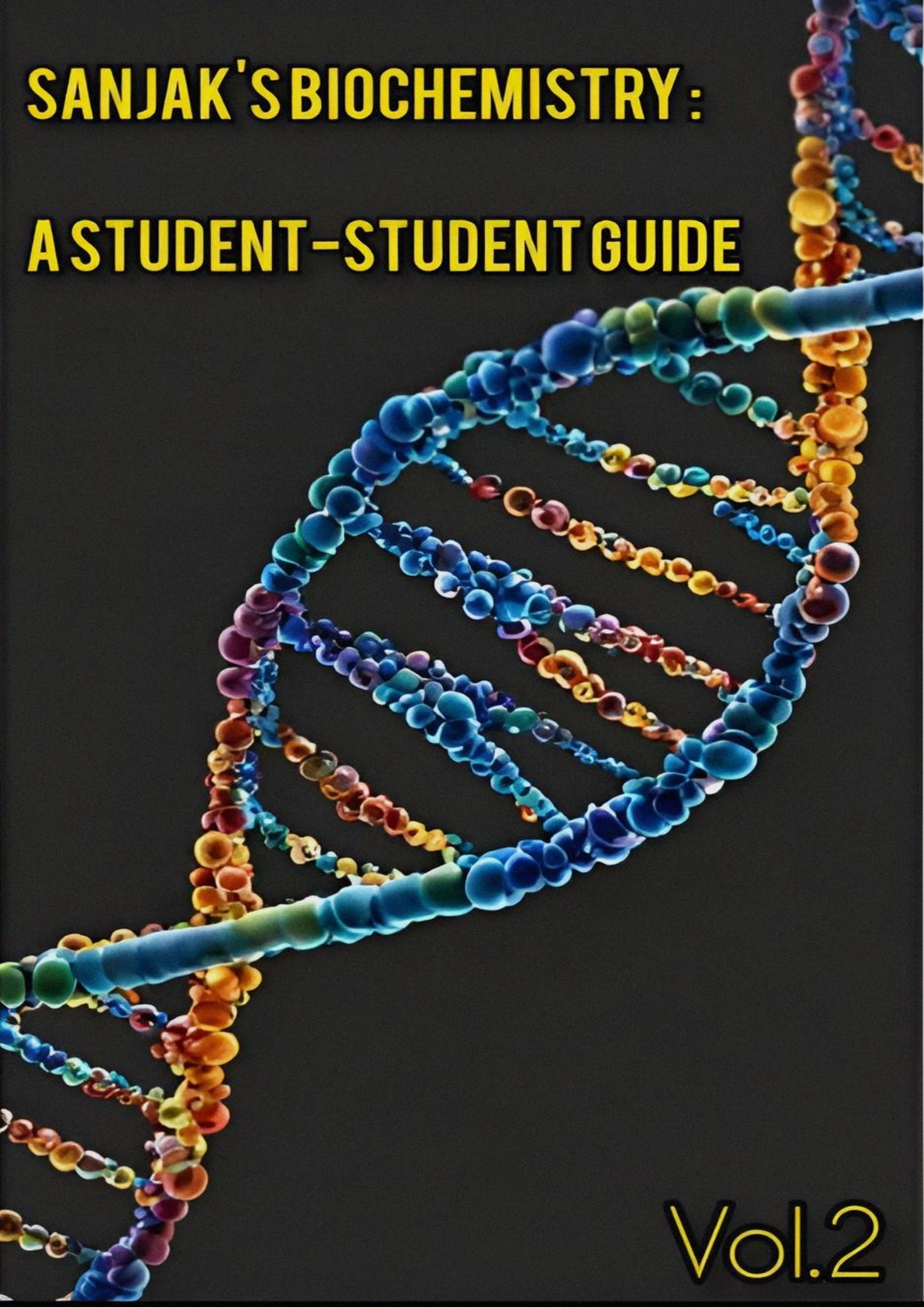
SANJAK'S BIOCHEMISTRY:

A STUDENT-STUDENT GUIDE



Vol.2

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

هذا العمل الذي بين يديكم و المسمى "Sanjak's Biochemistry" هو كتاب بايوكيميا موجه في المقام الأول للزملاء و الزملاء الاصغر سناً للطلاب بمختلف المجالات الطيبه كملخص مختصر لمادة الكيمياء الحيويه و النسخه التي بين أيديكم هي نسخه تجريبيه قد تحتوي على الكثير من الأخطاء اللغويه و البلاغيه التعبيريه ، فنرجوا منكم مساعدنا بال"feedback" و دعمنا لإصدار طبعات جديده اكثر تجويداً و تنظيماً و اكثر دقة عليميه .

هذا العمل ، والذي قام بعمله الطالب أحمد عبدالعظيم علي السنجك الطالب في كلية الطب و الجراحه بجامعة الشيخ عبدالله البدري والذي كما تم ذكره هو نسخه مبدئيه قد تحتوي الكثير من الأخطاء اللغويه و الاملائيه نظراً للأستعجال في انتاجه و سنعمل في الطبعات القادمه على تلافي هذه الأخطاء ، فقط عليكم بدعمنا و مساعدتنا عن طريق الـ "feedback" ، و نرجوا أن ينال هذا العمل المتعوب عليه إستحسانكم.

للحق و الحقيقه ما كان هذا الكتاب ليتم لولا مساعدة و مجهود الأخ و الزميل أمجد أحمد محمد الرشيد الذي كتب فصل "plasma proteins" ، إضافةً إلى مساهمته بالكثير من المعلومات و النصوص و البحوث و اتقدم له بالشكر

و نتقدم بجزيل الشكر و العرفان للأساتذة الأجلاء بشعبة الكيمياء الحيويه :

أ/عمر الطيب

د/ الحاج حمد بله

د/ ساره

تتسابق الكلمات، وتتزاحم العبارات، لتنظم عقد الشّكر الذي لا يستحقه إلا أنتم، إليكم يا من كان له قدم السبق في ركب العلم والتّعليم، إليكم يا من بذلتم ولم تنتظروا العطاء، إليكم أهدي المحتوى مشمول بالشّكر والتّقدير.

إن قلت شكراً فشكري لن يوفيكم حقّكم، حقاً سعيتم فكان السّعي مشكوراً، وإن جفّ حبري عن التّعبير يكتبكم قلب به صفاء الحبّ تعبيراً .....

شكر خاص :

شكر خاص لكل افراد أسرة عبدالقادر بلال و حاج اسماعيل و اسرة أبو القاسم البشير "شيخ العرب" بالقدواب مدينة بربر

و الشكر موصول ايضا للاصدقاء و الإخوه بكلية الطب و الجراحه جامعة الشيخ عبدالله البدري

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Chapter 1 Molecular biology

Introduction

In simple molecular biology is the study of structure, function and relationships between macromolecules " nucleic acids and protein" . The study of molecular biology is important in medicine and therapeutics , development in molecular biology is already leading to a new generation of synthetic and biotechnology-based approaches for the diagnosis and treatment of a variety of health-related diseases.

Nucleic acids

The nucleic acids are isolated at the first time from nucleus -of sperm of salmon- in 1869 by Fredric Miesher . He gave its first name as nuclein "now known as nucleic acids" , later the world start to understand that the DNA is the carrier of genetic information.
In 1953 James Watson and Francis crick discovered the double helical structure of the DNA molecule "based on the discoveries made by Rosalind Franklin " and They received Nobel Prize for it.

Beside DNA, the RNAs are not less important nucleic acids and they have many functions , however the structural and functional differences between DNA and RNAs will discussed later

Protein

Proteins are the active particles in the cells , they perform a vast array of functions within organism such as catalyzing enzymatic reactions, transporting water-insoluble compounds in the fluid compartments of the body, enter in the formation of extracellular matrix, provide the cell structure , cell signaling and response to stimuli, and several other functions .

Believe or not ; the traits of an organism " human in this case" such as eye color , hair color ,skin color, length, weight .....etc ; all are result from the action of specific proteins , and genetic diseases are result from alterations in specific proteins , in fact alteration in a single amino acid in specific proteins may be a life threatening condition as we will see later in this chapter Until the picture becomes clear.

Topics that will be discussed in this chapter

- Nucleotides structure and function
- Nucleic acids structure and function
- DNA & DNA replication
- Transcription
- Translation
- DNA damage and repair
- Cell cycle and cancer
- Regulation of gene expression

My dear brother , You can find these topics under the title genetics , molecular genetics or others ... Please We are not here .In order to stir up controversy and to differentiate between genetics or molecular biology and biochemistry and what is the best title, I hope you like this book .

Summary of nucleotides chemistry

Nucleotides are the building blocks of nucleic acids, beside they are serve as building blocks of nucleic acids the nucleotides have many other functions as we'll see .
The general structure of nucleotides is Nitrogenous base-sugar-phosphate .

Nitrogenous bases

Nitrogenous bases are nitrogen containing biological compounds .

There are two classes of nitrogenous bases

A. **Purine bases**
B. **Pyrimidine bases**

A. Purines

Normally we have two purines enter in the structure of nucleotides that form our nucleic acids

-Adenine **A**
-Guanine **G**

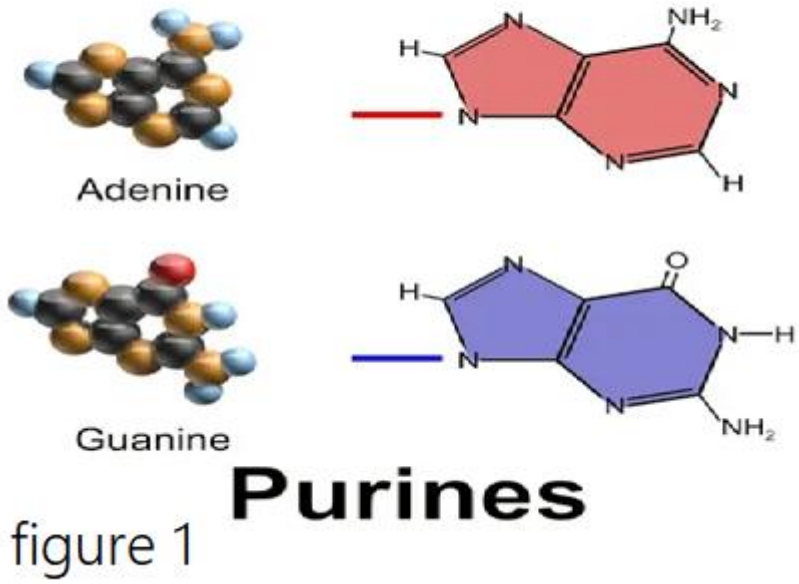


figure 1

Note that traces of unusual modified purines such as 7-methylguanosine might be found normally in our nucleic acids

B. Pyrimidines

Normally we have three pyrimidine bases enter in the structure nucleotides that forms our nucleic acids:

Uracil
Thymine
Cytosine

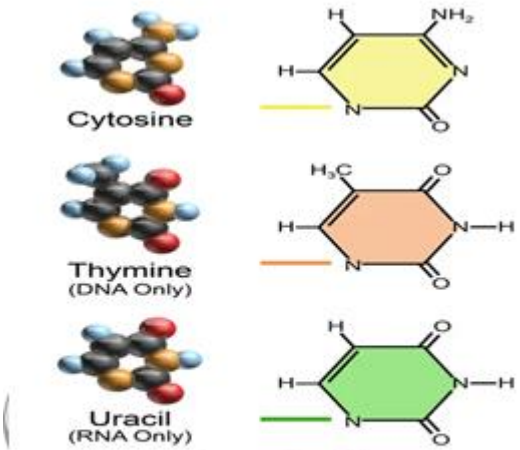


figure 2

Also nucleic acids contain traces modified Pyrimidnes such as psudouracil of unusual as we will see later

Note the the uracil is exclusively found in RNA while Thymine exclusively found in DNA

Remember this fact by using this text :

If they chose me between DNA and RNA I will choose RNA ; because it contains some of **U**

Sugar

Either ribose or deoxyribose (figure3) is a sugar that enter in the formation of nucleotides

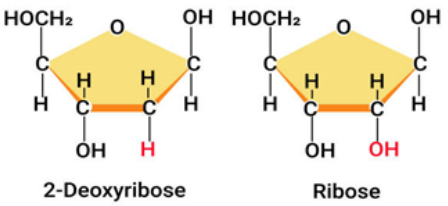


figure 3

-Ribose is found in the RNA while deoxyribose "deoxy at the 2`position " is found in DNA . Both ribose and Deoxyribose are in the D-form .

Phosphate

One, two or three phosphate group can exist in nucleotides , thus a nucleotide can be mono,di or triphosphate nucleotide (figure 4)

figure 4

Base-sugar & sugar-phosphate bonds :

A. Base-sugar : C1 of sugar "ribose or deoxyribose" linked with N1"pyramidine" or N9"purine" by β-N-glycosidic linkage (figure 5)

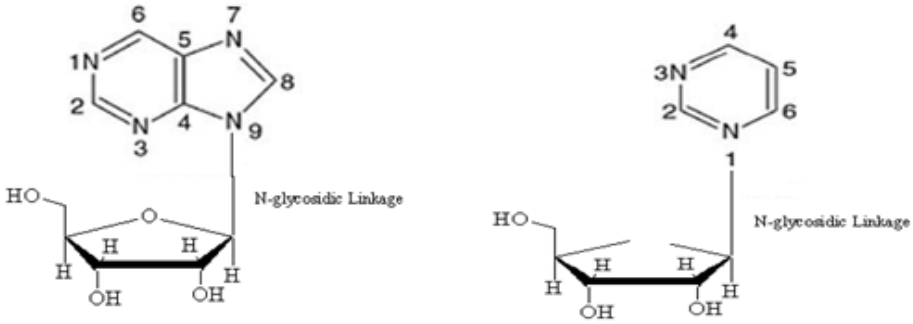


figure 5

Note that the complex of base with sugar is called nucleoside , thus we can define the nucleosides as derivatives of purine and pyrimidine that have a sugar linkage to a ring nitrogen of purine or pyrimidine (figure 6) .

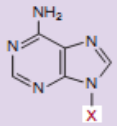
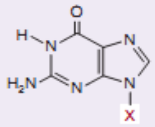
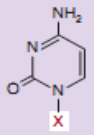
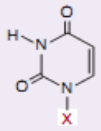
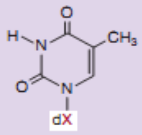
Purine or Pyrimidine	X = H	X = Ribose	X = Ribose Phosphate
	Adenine	Adenosine	Adenosine monophosphate (AMP)
	Guanine	Guanosine	Guanosine monophosphate (GMP)
	Cytosine	Cytidine	Cytidine monophosphate (CMP)
	Uracil	Uridine	Uridine monophosphate (UMP)
	Thymine	Thymidine	Thymidine monophosphate (TMP)

Figure 6

B. Base-phosphate linkage

phosphate linked to OH group of C5` by ester bond (figure 7) . Position 3` is also available to links with phosphate

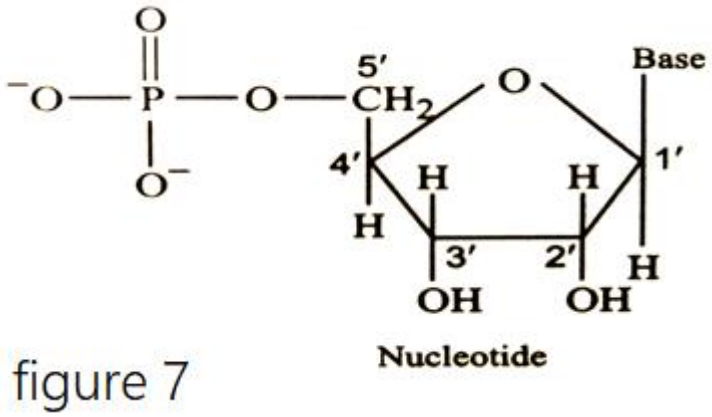


figure 7

Remember that the nucleotides consist of Base-sugar-phosphate .

Nomenclature of nucleotides

Nucleotides are named by adding mono\di\tri phosphate to the end of the name of corresponding nucleosides (figure6)

Note that if the sugar is deoxyribose , nucleotide will named deoxy nucleoside phosphate" mono/di/triphosphate

Functions of nucleotides :

Nucleotides have many other functions beside nucleic acids precursors , examples for these functions:

- Energy currencies such as ATP
- cAMP and cGMP act as 2nd messengers (Figure 9)
- Phosphoadenosyl phosphosulfate "PAPS" is an active sulfate donor in many reactions such as sulfate conjugation reactions of drugs(figure 10)
- S-Adenosyl methionine is methyl donor in methylation reactions (Figure 11)
- AMP is VD for coronary arteries
- CTP participate in biosynthesis of phospholipidsUTP react with monosaccharides glucose and galactose and activated them into UDP-glucose and UDP-Galactose respectively .
- Nucleotides share in the structure of many Coenzymes " NAD, FAD, NADP and Coenzyme A .
- structure of many Coenzymes " NAD, FAD, NADP and Nucleotides share in the- . Coenzyme A

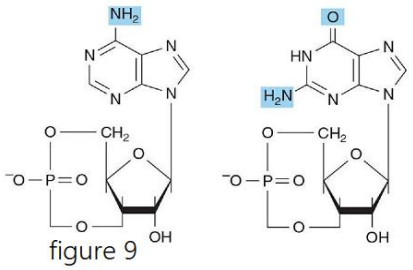


figure 9

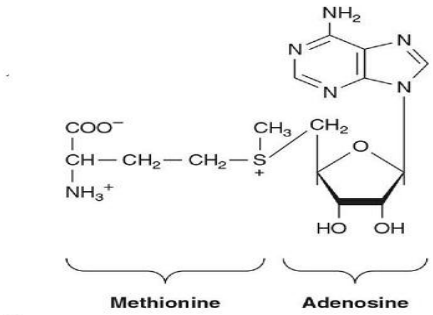


figure 11

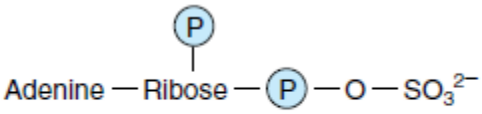


figure 10

Notes

Nucleic acids contain "in addition to A,G,C,U and T" traces of unusual modified bases such as 5-methylcytosine and others as we'll see later

.

Synthetic nucleotide analogs have numerous applications in clinical medicine:

-Allopurinol used in treatment of hyper uricemia and Gout (see purine metabolism)

-Others used in chemotherapy of cancer and viral infections

-Azathioprine used in organs transplantation to suppress immunologic response

It may be difficult for you to understand the mechanism now , and if the mechanism discussed here , this will cause a confusion.

Nucleotides that exist in the nucleic acids are found in the monophosphate form .

Nucleic acids

Nucleic acids are polymers of nucleotides linked through phosphodiester linkages ,in chain-like fashion. Two types of nucleic acids; deoxy ribonucleic acid "**DNA**" and Ribonucleic acids "**RNAs**" are presence in the cell, together constitute about 15% of the cell dry weight.
The DNA acts as genetic material that encoded mainly for proteins which allow all forms of life to function, reproduce and grow .

The RNA comprises of several types , all are synthesized from DNA and play a vital role in protein synthesis.

Earlier we talked about nucleotides and their structure and now we saying that the nucleic acids are polymers of nucleotides ,how these nucleotides linked together?
Nucleotides of both DNA and RNAs are covalently linked by **phosphate group**, in simple term the phosphate group links between third and fifth carbons "of sugars" of two successive nucleotides (figure 12).

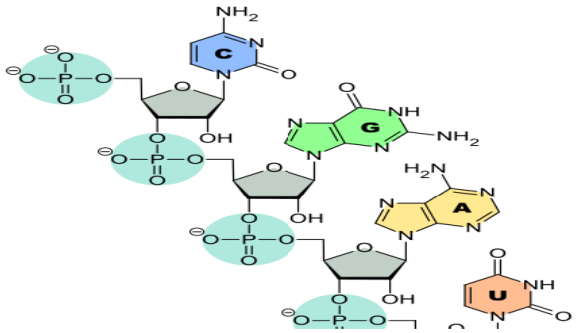


figure 12

Thus the linear polynucleotide chain has "polarity" i.e distinct 5'end and 3'end .
The 5` end has a free p group while 3` end has a free oh group (figure 13).

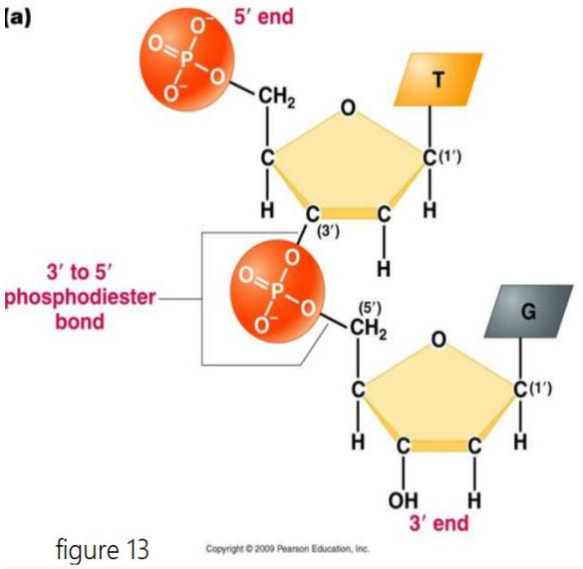


figure 13 Copyright © 2009 Pearson Education, Inc.

DNA

Previously we talk about that the DNA is the carrier of genetic information ,to be honest with you it is difficult to understand this point "now". But we can say that the genetic information presence in the sequence by which nucleotides "in the deoxy form" arranges within the DNA molecule , in fact the information presence in the sequence of nitrogenous bases because the phosphate and deoxy ribose are identical in all nucleotides of DNA .
However This information are just a" constructions " for the synthesis of Proteins and RNAs

DNA structure

James Watson and Francis crick in 1953 described the three dimensional structure of DNA ,its consist of two polynucleotide chains "strands" run in opposite directions "antiparallel" . Nucleotides of the same strand "polynucleotide chain " linked together by **3`-5` phosphodiester** bonds and they linked with nucleotides of the other strand by **Hydrogen** bonds result from the chemical interaction between nitrogenous bases of the opposite strand (figure13) .The structure of the DNA looks like a ladder , the backbone of this ladder consist of phosphates and sugars "deoxyribose in this case" which they represent the hydrophilic part , and stairs of ladder consist of the nitrogenous bases that linked with correspondings in the opposite strand (figure 14) .This ladder is twisted around common axis(figur 14) .this twisting result in major and grooves(figure 14) and we will see their function

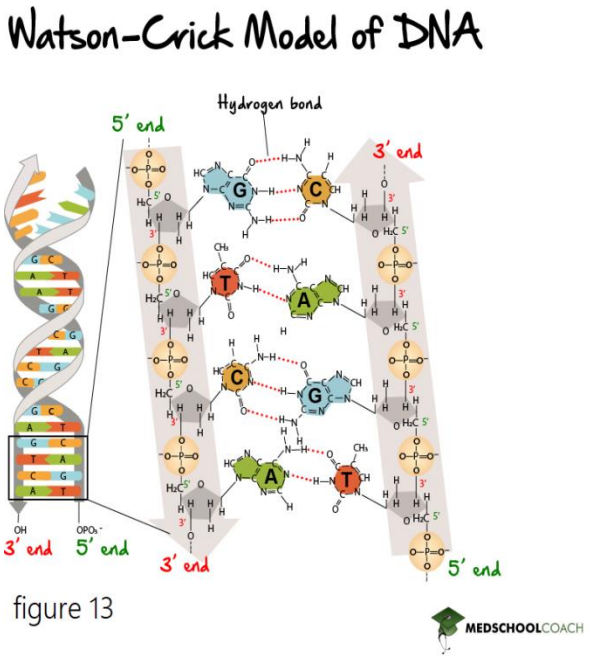


figure 13

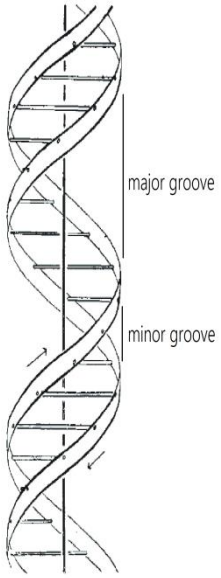


figure 14

The watson-crick structure also known as β form which is the most common form, and usually exist under the physiologic condition ,it said to be right-hand helix . In the test tube at least there are six forms "A-E" and it's not necessarily to know about the differences between this forms

Base bairing rules "chargaffs rules"

The bases of one strand are linked with the bases of the corresponding strand, but this linkage not randomly... normally Adenosine always pairs thymine and guanosine always pairs cytidine .That's means the adenine content equals thymine content and guanosine content is equal to cytidine contact in the DNA "pyrimidines =purines" (Figure 15).

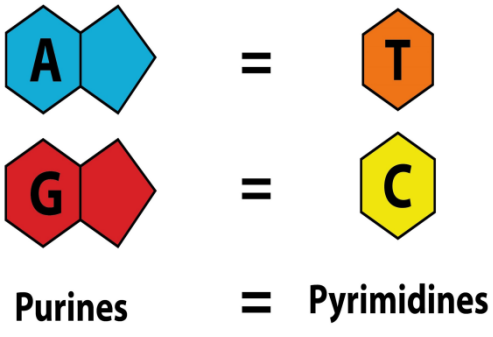
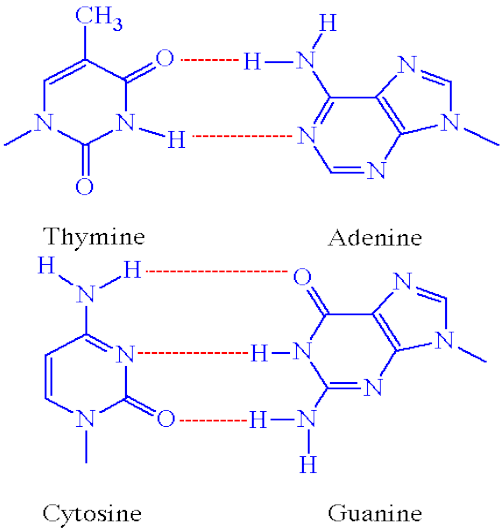


figure 15



Functions of major and minor grooves :

As far as I know the major function of this grooves is to provide a partition for interaction with specific proteins ,this important in replication ,transcription and also gene regulation

DNA organization

In humans each somatic cell contains about six billions base pairs in the nuclear DNA "diploid number of chromosomes" .while the gametes contain about three billions base pairs "haploid number" , however a one base pair is around 0.3 nm in length "0.34×10^{-9}" thus the somatic cell contains about two meters of DNA [$(0.3\times10^{-9})\times(6\times10^{9})$]and gametes contain about one meter of DNA .... note that the radius of nucleus is about 10μ . How this two meters "or one" residue in this little space ? The DNA is highly condensed and organized inside the nucleus , in fact these two "or one" meters not exist continuously , but it divide between 23 pairs "or 23" linear DNA units "chromosomes" (figure 16) .

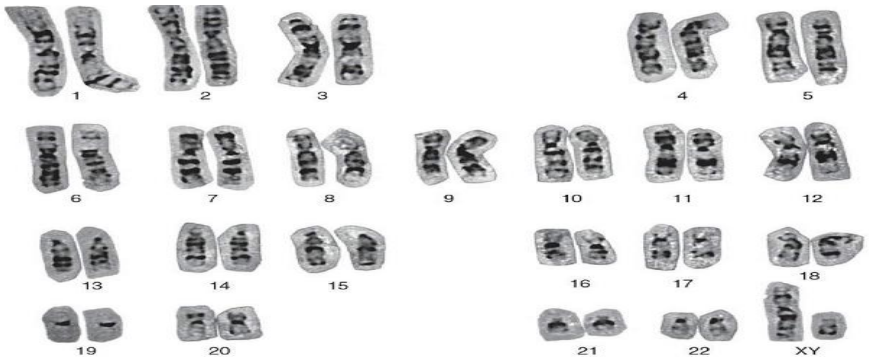


figure 17

Chromosome

Chromosome is a nucleoprotein , composed of nucleic acids "mainly **DNA**" and basic proteins "mainly **histones**" , chromosome also contain some RNAs and other non-histone proteins . However chromosome is a very condensed, very organized DNA .

The chromosomal material said to be **chromatin** . Chromatin is a chromosomal material which formed by DNA-histones complex "mainly" . Short segment of The **negatively** charged DNA "due to it's phosphate content" wrapped around positively charged histones. This wrapping called **nucleosome** which is the organizational unit of the chromatin (figure17)

DNA is linked by ionic bonds with at least five types of histones H1,H2a,H2b,H3 and H4 . The H2a,H2b,H3 and H4 together form an octameric molecule i.e two molecules of each H2a,H2b,H3 and H4 aggregate to form octamer . However these octamer wrapped by segment of DNA "145 bp" 1.75 time .However the DNA-octamer complex is called **nucleosome core** . The H1 appear to bind to the DNA between nucleosome beads (figure 18)

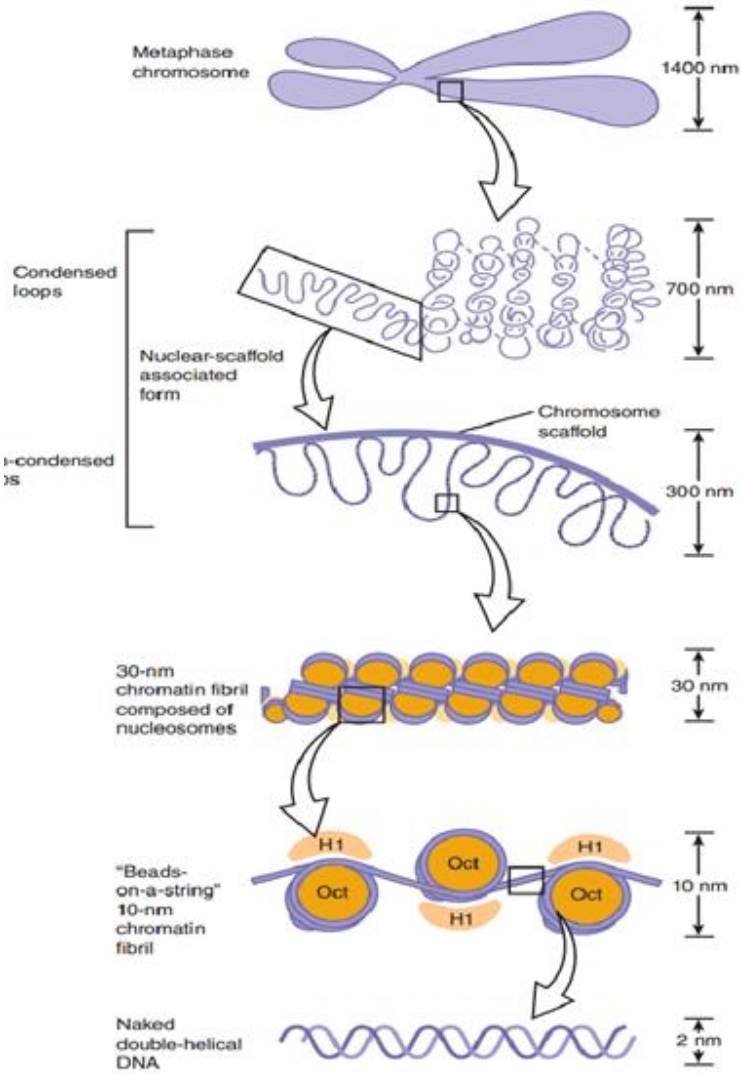


figure 17

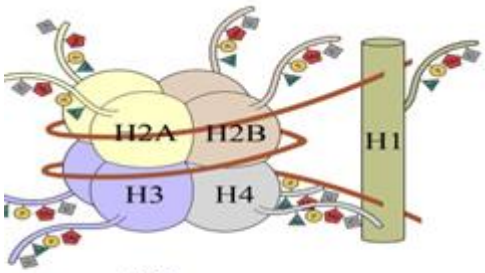


figure 18

Genes

Gene is a segment of DNA , is DNA sequences that contain genetic information "instructions" either for synthesis a functional polynucleotide or RNA .
Mitochondrial DNA '
Beside nucleus DNA is also found in the mitochondria. Mitochondrial DNA is a circular DNA found inside the mitochondria . In humans mitochondrial DNA spans about 16,500 bp which represent a small fraction of the total DNA content of the cell .
MtDNA inherited only from the mother why?! To answer this question we must return to the Embryology. the mitochondria of sperms lie in the neck of the sperm , and during fertilization the only part of the sperm that enters ovum is head of the sperm .that means the sperm only share by it nuclear DNA in fertilization . This explains why some hereditary diseases are only inherited from the mother and do not obey Mendel's laws "mitochondrial non-Mendelian diseases.".

Mitochondrial DNA contains 37 genes , all of which are essential for normal mitochondrial functions , 13 of this genes provide instructions for synthesis proteins that involved in oxidative phosphorylation, and the rest for synthesizing RNAs which are important in protein synthesis

RNA

With the discovery of the DNA double helix , researchers turned to the structure of RNAs as next critical puzzle to be solved in the road to understanding the molecular basis of life .
RNA consist of 4 nucleotides" in the monophosphate form " A,G,U and C and also traces of unusual modified bases as we will see

The typical RNA molecule is single strand but some RNAs folded on themselves and show some sort of double strand like characters (figure 20) .Double stranded RNAs "precursors" are also found .

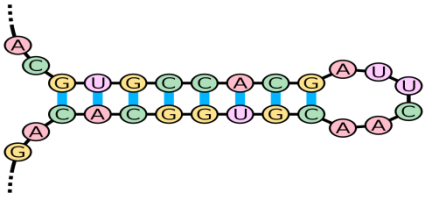


figure 20

There are 3 major types of RNA

mRNA

Jacob and monod "1961" proposed the name messenger RNA for the RNA that copy and carry information "instructions " for protein synthesis from **nucleus** "site of DNA" to the site of protein synthesis "granulated endoplasmic reticulum " .
mRNA always single strand . Contain 7-methylguanosine cap "modified base" in the 5` end and poly A tail at the 3` end (figure21) . Note that before the poly A tail there is an Adenosine rich sequence which is a signal for terminates transcription "see transcription "

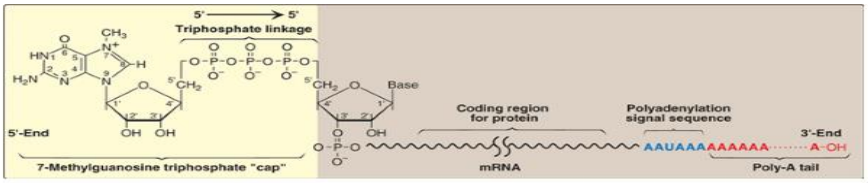


figure 21

Transfer RNA "tRNA"

essential component in the protein synthesis process .tRBA serve as amino acid carrier .thus there are at least one specific tRNA for each amino acid "20 amino acids enter in the formation of proteins " . Several models for structure of the tRNA have been proposed, however the cloverleaf model is the most widely accepted (figure 22)
tRNA consist of :
-**D lope** : it called D lope because it contains unusual modified base "Dihydrouracil" (figure 23A).
-Anticodone loope : pairs with complementary sequence on the mRNA molecule, ensuring that the appropriate amino acid is inserted into the protein being synthesized as we will see in protein synthesis

-TuC loop : contains unusual modified base pseudo uracil (figure 23 B)

-**Extra arm** or **variable loop** : most variable feature in tRNA
Finally there are special sequence in all tRNAs in the 3` end which is CAA , the amino acids linked with OH of the A in 3` end (figure 22)
In contrast to other RNAs the tRNA contain a significant amount of unusual bases "about 15%"

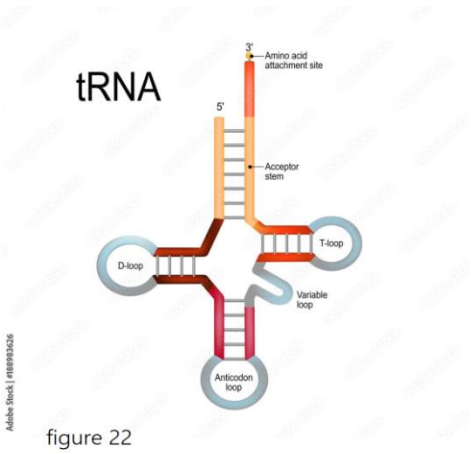
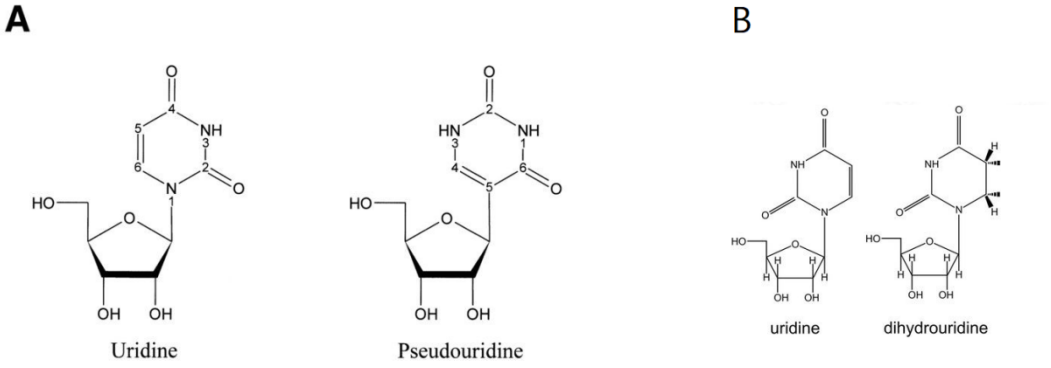


figure 22



rRNA

As it's name suggests ,is found in ribosomes ,it is found in association with different proteins as component of ribosome . It comprise about 80% of the total RNA content in the cell .rRNA also contain some unusual bases .

In eukaryotic cells there are four distinct size species of rRNA" 28s ,18s,5.8s and 5s "

While prokaryotes contain only 3 distinct size " 23s ,16s and 5s"

Note : the S in 28s for example stand for svedberg unit which is a sedimentation unit ,related to molecular weight and shape of compounds

MORE DETAILS WILL BE AVAILABLE IN THE NEXT EDITION

Nucleotides metabolism

Dietary nucleotides

Because nucleic acids are presence in all cells our diet thus rich in nucleotides, but this dietary nucleotides All most are not utilized by the body ; instead there are digested, catabolized and excreted mainly with stool and to little extent in urine .
By the action of DNAase and RNAase nucleic acids are digested to produce mononucletides . This mononucletides may absorbed as such , or undergo further digestion to yield free purine and pyrimidine bases which in turn are degraded then may absorbed and excreted in urine, however little or no dietary purines and pyrimidines is incorporated into tissue nucleic acids. Our tissues able to synthesize their requirements of nucleotides.

Purine metabolism

Purine biosynthesis

There is two separate mechanisms for synthesis of both purines and pyrimidines :
1. **Denovo** synthesis "from pits and part"
2. **Salvage** mechanism " recycling a partially degraded nucleotides "

1. denovo purine synthesis

We don't synthesize a free purine ring , but we build the purine base upon ribose phosphate, in other words we synthesize a purine nucleotides and not purine base

Steps

Denovo purine synthesis begins with the precursor molecule **Ribose 5 phosphate** "R5P" . R5P converted to phosphoribosyl pyrophosphate "**PRPP**" this step catalyzed by PRPP synthetase .
In next step amide of glutamine displaces the pyrophosphate of PRPP by the action of enzyme **PRPP amidotransferase** , this step is the commited step in the purine de novo synthesis
In the next nine steps " not necessarily to know about it" a series of reactions that require Aspartate , glycine, THF and glutamine are occur , and ultimately lead to formation of **inosine mono phosphate** "IMP" which is the first synthesized purine nucleotide and the precursor of AMP and GMP (figure 24)
IMP then can converted into AMP and GMP (figure 25)

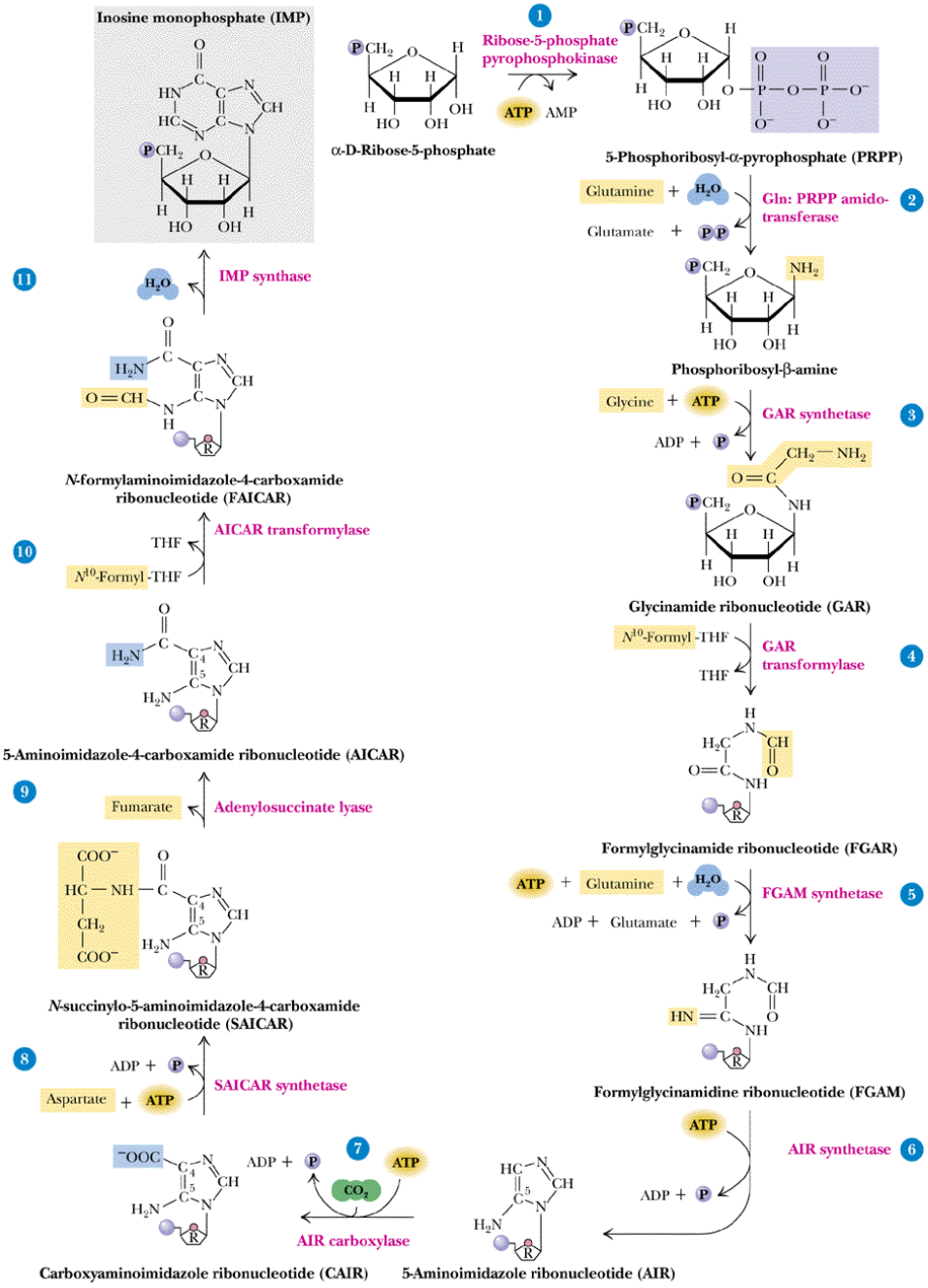


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Figure 24

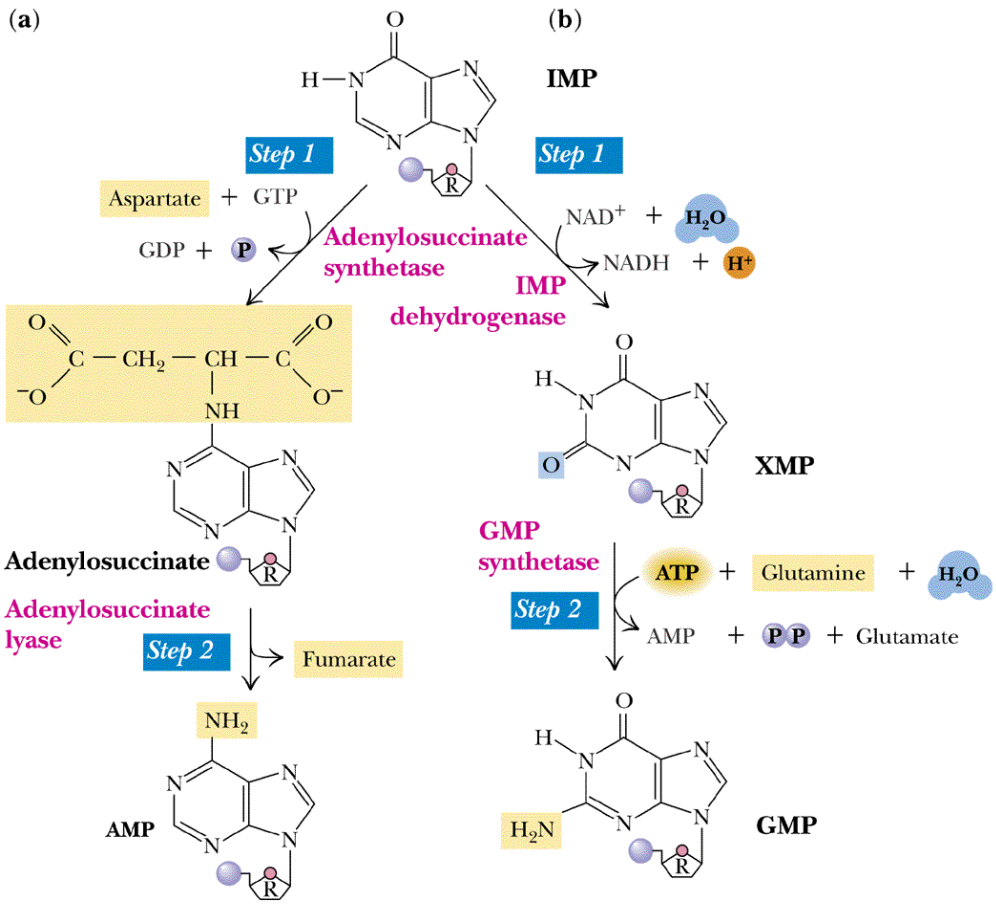


figure 25

Note that the GTP is required for AMP synthesis , and ATP is required for GMP synthesis

Regulation of denovo purine synthesis

Regulation of IMP synthesis:

Allosterically regulated at the first two steps

-R-5-P pyrophosphokinase:

Inhibited by ADP & GDP

-phosphoribosyl pyrophosphate amidotransferase :

Inhibited by A “series”: AMP, ADP, and ATP .

Inhibited by G “series”: GMP, GDP, and GTP.

PRPP is “feed-forward” activator.

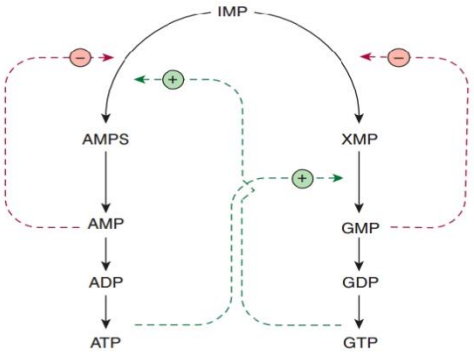


figure 26

Regulation of AMP and GMP synthesis

-AMP synthesis:

Adenylosuccinate synthetase is feedback-inhibited by AMP

-GMP synthesis:

IMP dehydrogenase is feedback-inhibited by GMP

Salvage purine pathway

In simple meaning it done by recycling partially degraded purine nucleotides (Figure 27)

" A" in figure 27 is the most important and we will discuss it

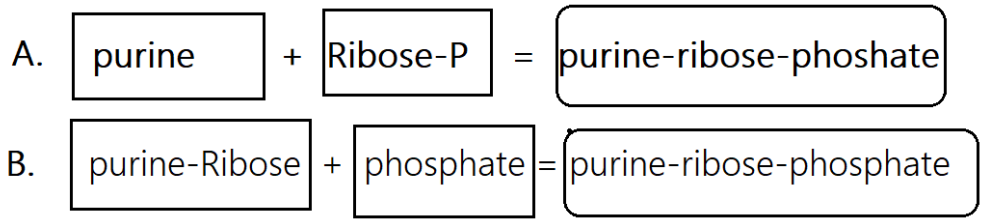


figure 27

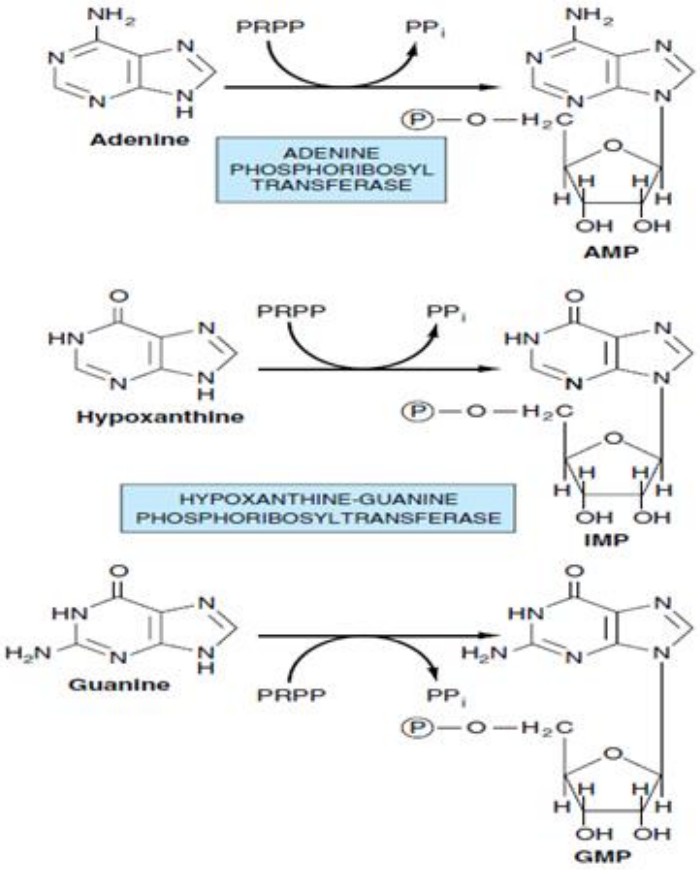
Steps

Purine salvage begins with free bases , **hypoxanthine**, **guanine** and to little extent **adenine**

Hypoxanthine and guanine are combined with PRPP by the action of same enzyme , guanine converted to GMP while hypoxanthine form IMP in which can then converted to AMP . Both this salvage reactions are catalyzed by HGPRTase "hypoxanthine guanine phosphrybozyl transferase" . Defect in HGPRTase cause lesch nyhan syndrome also called choreoathetosis self-mutilation syndrome " see clinical later"

A less common purine salvage pathway begins with adenine, adenine combines with PRPP , this catalyzed by APRTase. (Figure 28)

figure 28



Note that that salvage reactions critically important in brain

Degradation of purines

Degradation of purine nucleotides start with hydrolytic removal of their phosphates to yield the corresponding nucleosides .

Nucleosides then cleaved into free bases and Ribose1 phosphate by purine nucleoside phosphorylase "phosphorylation of ribose at 1` position to cleave the linkage between the base and ribose " . Adenosine is poor substrate for this enzyme, thus it must to be converted into inosine which is appropriate substrate for purine nucleoside phosphorylase .

Uric acid is end product of purine catabolism in humans. It synthesized by the action of xanthine oxidase (figure 29)

Precipitation of uric acid crystals in joints lead to Gout "see later"

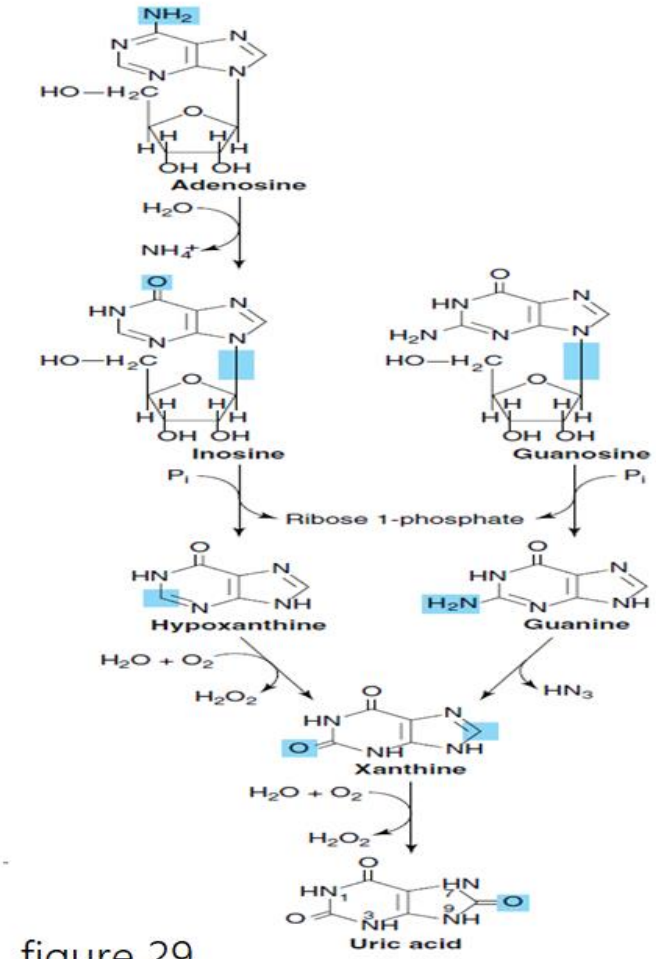


figure 29

Synthesis of deoxy purines

Synthesis of deoxy ribonucleotides is required for DNA synthesis . Deoxy purine nucleotides synthesized from corresponding purine nucleotides, this process catalyzed by **ribonucleotide reductase** .ribonucleotide reductase reduce the ribose residue (figure 30)

Ribonucleotid reductase also acts on pyrimidines . The substrates of this enzyme are **ribonucleotides in** the **Diphosphate** form

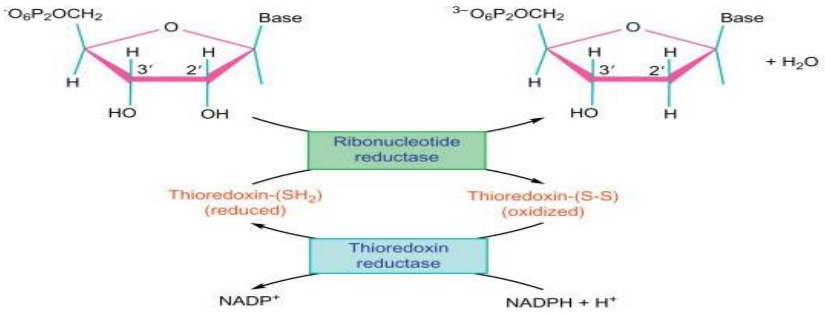


figure 30

Clinical

Lesch-nyhan syndrome

Also called choreoathetosis self- mutilation syndrome, was first described at john Hopkins hospital in 1964 by Michael **Lesch** and William **Nyhan** in two brothers with an unusual set of symptoms.

Lesch-nyhan syndrome is a genetic defect . X-linked disease, primary affect males , results from defect in HGPRTase gene which in turn result in defect in HGPRTase enzyme thus affect the purine salvage reactions (figure 32) .

Characterized by :

- Purine synthesis increase 200 fold
- Increase uric acid production
- Neurologic manifestations because salvage reactions important in the brain " brain has very low capacity for de novo synthesis " :
- Spasticity
- Neurologic defects
- Aggressive behavior
- Self-mutilation

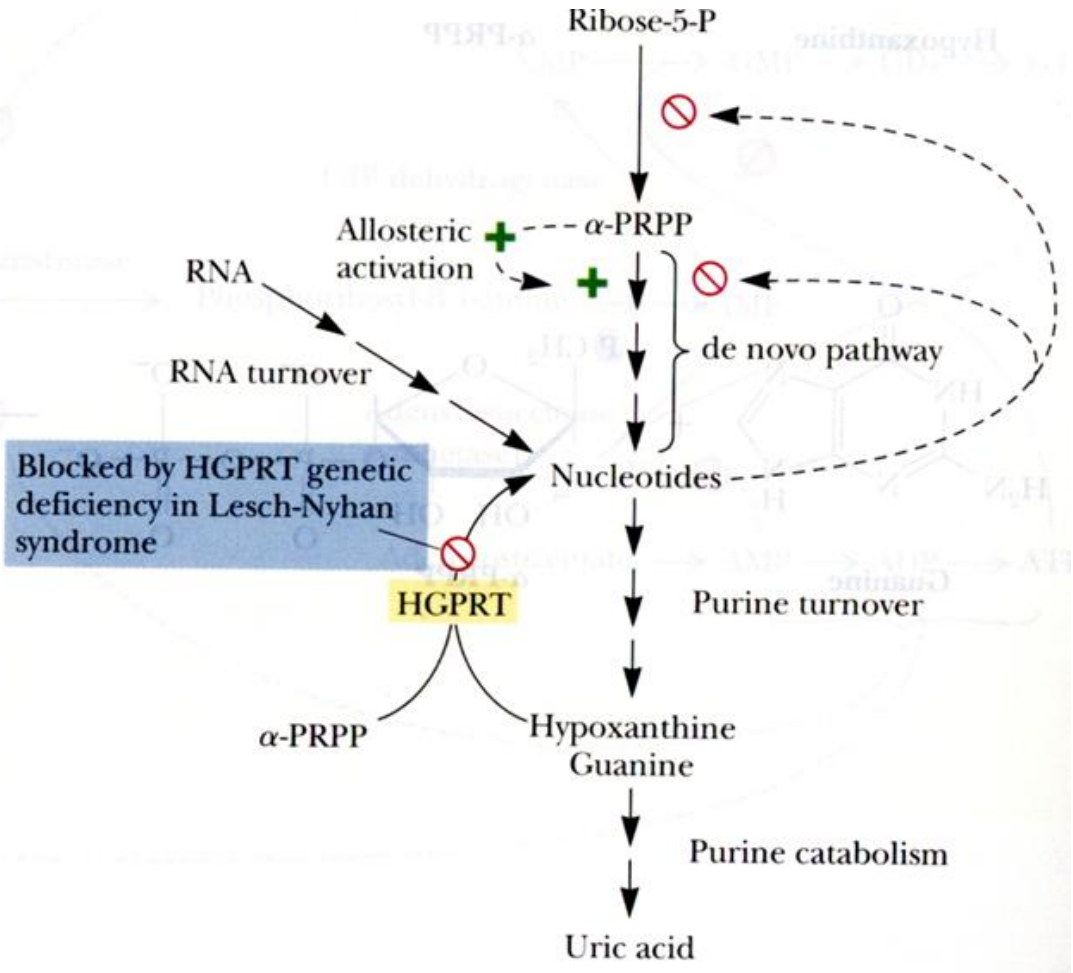


figure 32

Hyperuricemia

Condition in which serum urate level increase above the normal level ' about 2.7 mg/dl" and exceeds the solubility level of plasma . Some hyperuricemias reflect specific enzymes defects and others secondary to other diseases

Hypouricemia

Associated with xanothine oxidase deficiency or severe liver faliure in other cases
Patients with severe enzyme deficiency exhibit xanthineuria and xanthine renal stones

Gout "disease of kings "

The name gout is derived from the Latin word **"Gutta"** which means droplets , the name comes from ancient belief that it was caused by drops of humors "body fluids" spilling into the joints from the blood.
Gout is a type of **inflammatory arthritis** caused by deposition of urate crystals in joints, usually affects one joint "usually big toe" (Figure 33)

figure 33

Causes

-Various defects in PRPP synthetase present clinically as gout i.e increase PRPP synthetase affinity to R5P , or resistance of PRPP synthetase to the feedback inhibition , however this ultimately lead to over production of purines and in turn over degradation of purines .
- Von jerk disease "G6PD deficiency" (figure 34)
-Abnormalities in renal handling of uric acid
-Secondary to enhanced generation of PRPP precursor Ribose5p
-Life style "royalty class " i.e diet rich in meats and alcohol consumption

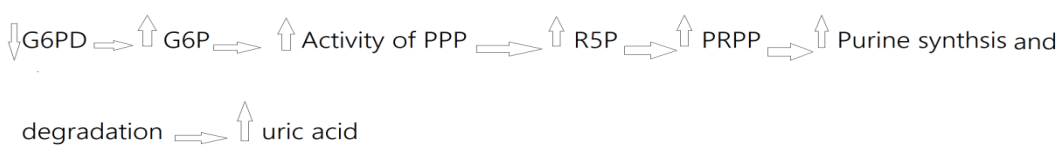


figure 34

Pathogenesis:

Elevated urate levels lead to deposition of monosodium urate crystals in joints , that **trigger** a strong inflammatory response cause acute gouty arthritis (Figure 35)

figure 35

Treatment

treat the inflammatory symptoms by NSAID , not aspirin. Aspirin not used because it decrease the renal excretion of urates.
Then treat hyperuricemia commonly by Allopurinol which inhibit xanthine oxidase
" competitive inhibition, it competes xanthine on the active site of xanthine oxidase "

ADA and PNP deficiency

Both are autosomal recessive diseases .Deficiency in one "ANA or PNP" lead to accumulation of intermediates , dATP and dGTP are members of this accumulated intermediates , DATP and dGTP can reach a toxic levels that inhibit ribonucleotide reductase and thus interfere with formation of deoxyribobucleotides and this depletes the cell from DNA precursors , thus DNA synthesis and cell division are affected. this found specially in lymphocytes , thus deficiency in either ANP or PNP are one of the causes of Severe Combined Immunodeficiency **SCID**
Note : PNP deficiency show normal B lymphocytes or variable degrees of dysfunction

Pyrimidine metabolism

Denovo pyrimidine synthesis

Unlike purines, pyrimidine ring synthesized before ribose is added . Pyrimidine synthesis begins with glutamine and carbondioxide ,which combine to form carbamoyl phosphate in reaction catalyzed by **Carbamoyl phosphate synthetase 2** "**CPS2** " .the (figure 36) shows a table illustrates the difference between CPS1 and CPS2 . **Carbamoyl phosphate** then combines with **aspartate** to ultimately form **orotic acid** .However orotic acid then react with PRPP to form **orotidine monophosphate** which is the first t synthesized pyrimidine nucleotide reaction catalyzed by orotate phosphoribozyl transferase . (Figure 37)

Differences between CPSI and CPSII :

	CPS I	CPS II
•SITE	Mitochondria	Cytoplasm
•Pathway of	Urea	Pyrimidine
•Positive Effector	NAG	------
•Source for N	Ammonia	Glutamine
•Inhibitor	--------	CTP

figure 36

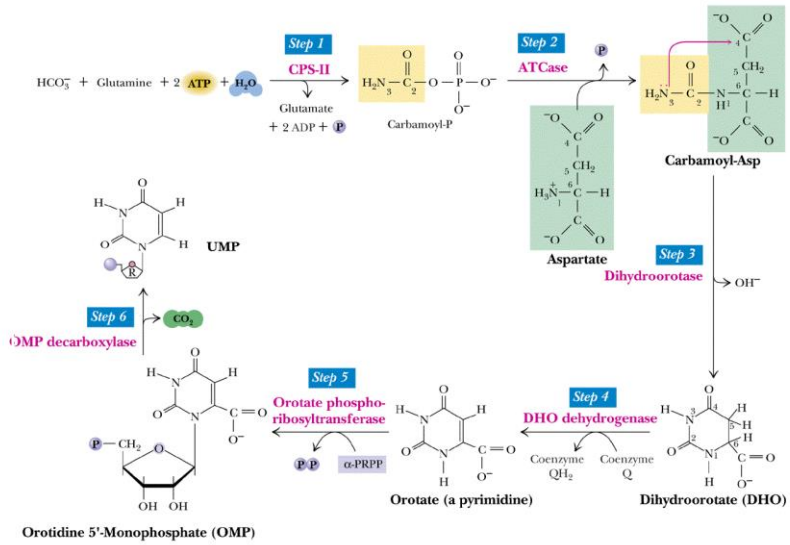


figure 37

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OMP then decarboxylated to form UMP this catalyzed by orotidyl decarboxylase . UMP then phosphorylated to UDP which has two possible fates :

1. Phosphorylated again to yield UTP , UTP then converted into CTP
2. Undergoes reduction to yield dUDP , then dUDP undergos methylation by **thymidylate** synthetase "an enzyme require **methyline-THF** as co factor " to form dTMP (Figure 38)

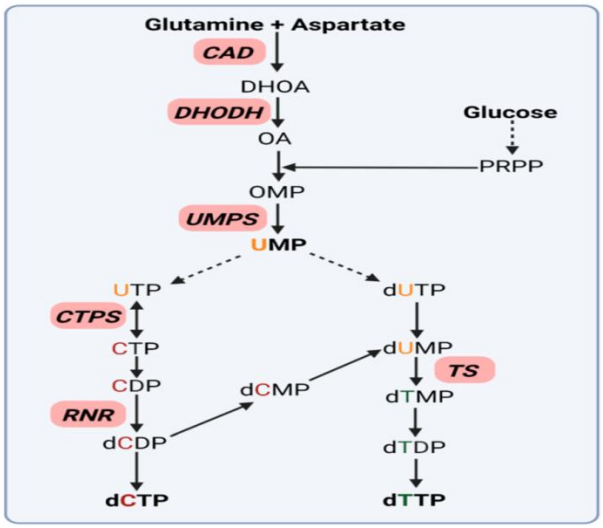


figure 38

Methotrexate blocks the reduction of Dihyrofolate that illustrated in(figure 39). it used as anticancer

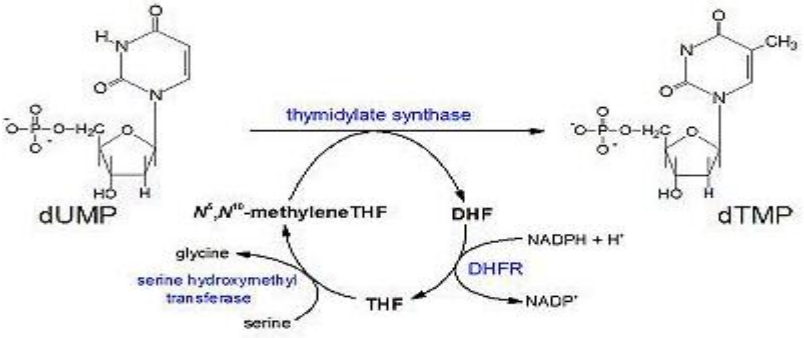


figure 39

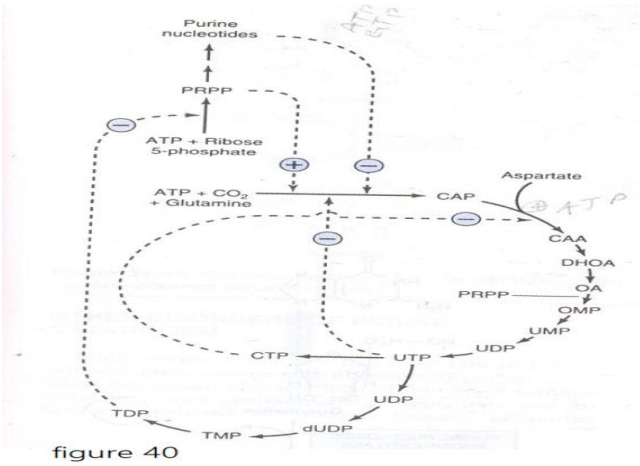


figure 40

Figure 40 : regulation of pyrimidine denovo synthesis

Pyrimidine salvage :

Mammalian cells reuitilze few free pyrimidines , salvage reaction converts the pyrimidine nucleosides into their respective mononucletides (Figure 41)

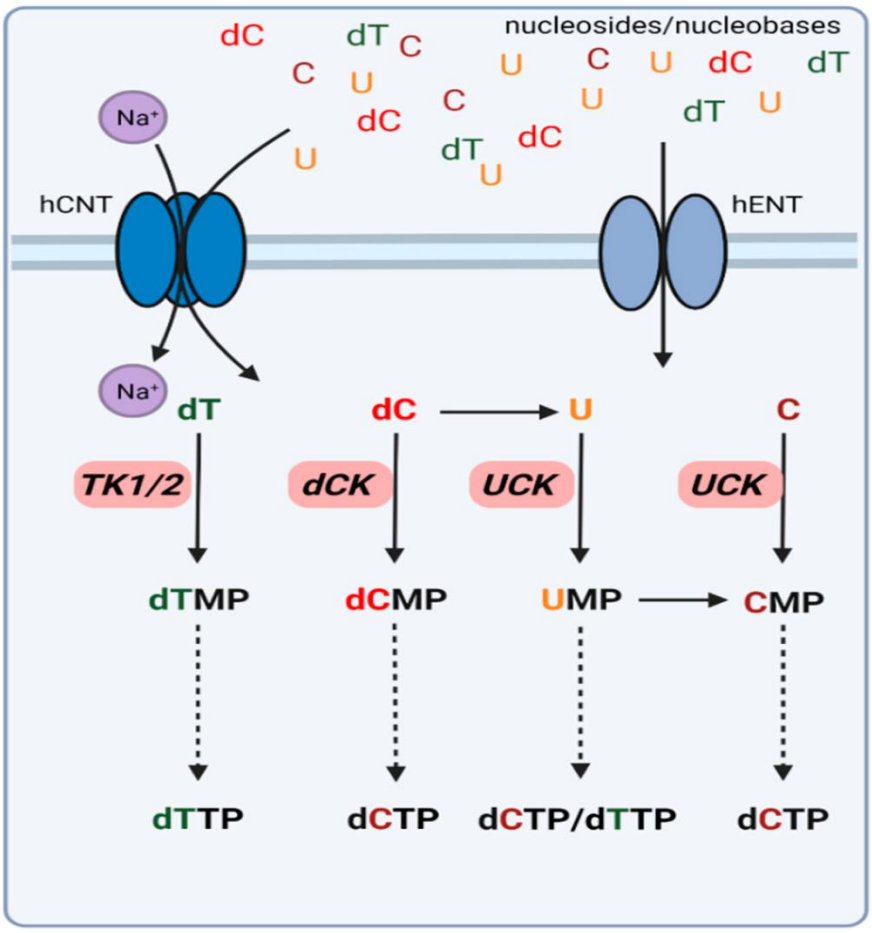
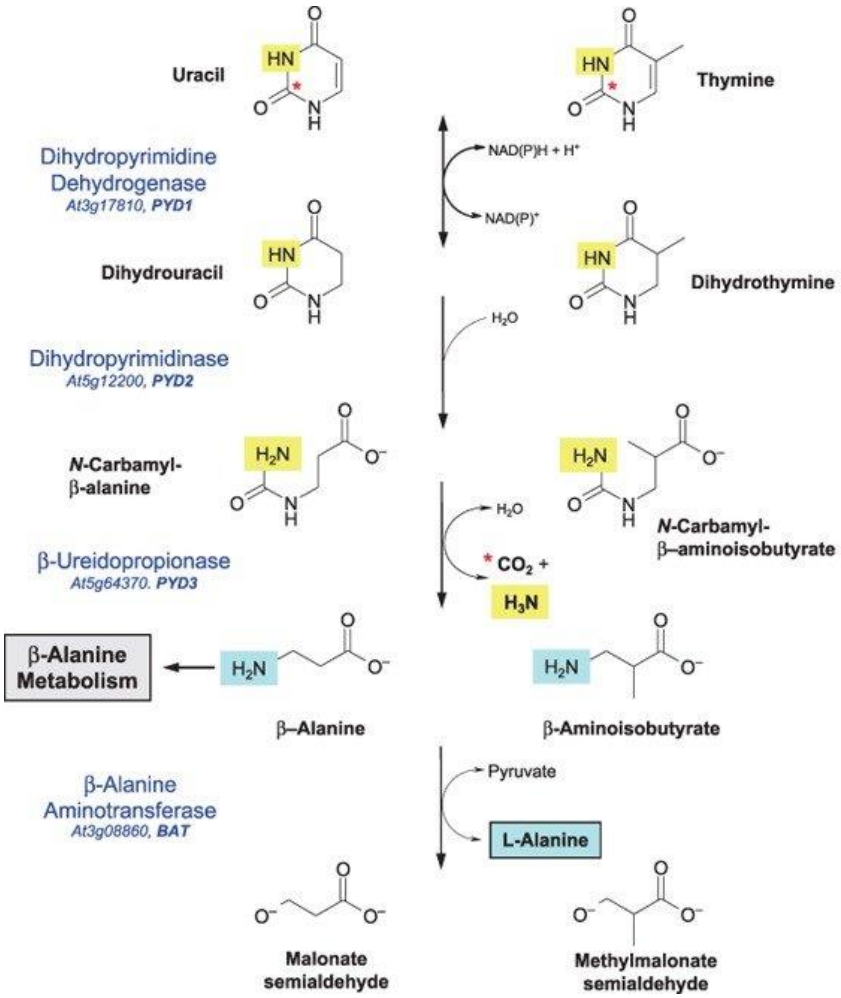


figure 41

Catabolism of pyrimidines

-In humans, pyrimidines are recycled from nucleosides (via phosphoribosyltransferase), but free pyrimidine bases are not salvaged
-Catabolism of cytosine and uracil yields β-alanine, ammonium, and CO_2
β-alanine can be recycled into the synthesis of coenzyme A
-Catabolism of thymine yields β-aminoisobutyric acid, ammonium, and CO_2



Hereditary orotic aciduria

Deficiency of orotate phosphorybozyl trasferase and/or orotidylate decarboxylase, "which are two enzymatic activities of the same protein " lead to oroticacuria .
This is a rare condition characterized by megaloblastic anemia crystalline sediment of orotic acid in urine and poor growth ,the important cardinal signs are caused not by accumulation of orotic acid but by pyrimidine deficiency. Patient with hereditary orotic aciduria responsive to **oral uridine**
Some urea cycle enzymes deficiency "in the mitochondria of liver" may exhibit orotic aciduria , because **accumulated Carbamoyl phosphate diffuse into the cytoplasm and then can converted to orotic acid**
Note that many anti neoplastic drugs inhibit nucleotide metabolism

DNA replication

Living cells must be able to duplicate their entire set of genetic instructions every time they divide. The DNA replication is the process by which a DNA molecule copied into **two identical DNA molecules ,** we can say that replication is the process of producing two identical **replica** of DNA from one original DNA .
Both two strands of the DNA acts as templates for synthesizing a new "complementary" strands , these scenario known as **semi-conservative** i.e each daughter molecule is a hybrid double helix in which one strand is parental "conserved from the parent DNA molecule " and the other newly synthesized. This fact was discovered by Mathew meselson and Franklin stanl in 1958 , not necessarily to know about their experiment, but it has been called " the most beautiful experiment in biology " (figure 42). However the DNA synthesized by repetitive addition nucleotides to 3`end of the growing polynucleotide chain . i.e the direction of synthesis 5` to 3' (figure 48)

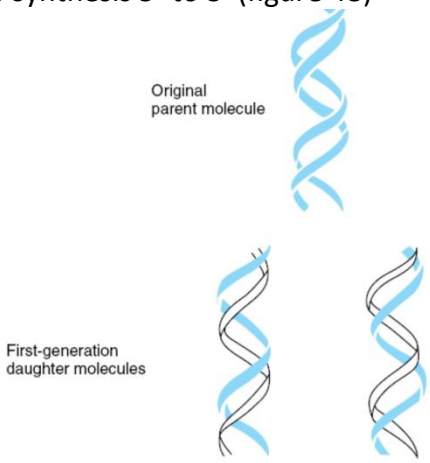


figure 42

The requirements

-dATP , dGTP , dCTP and dTTP , note that when we insert dNTP into growing chain we loss a pyrophosphate from dNTP .
-Enzymes and proteins are also needed for replication

Steps

1 strand separation : strand separation is the initial step in the DNA replication process . The two strands are separated by the action of enzyme called helix unwinding protein, also called " unwindase" and "helixase" . This enzyme breaks the hydrogen bonds in between the bases of two strands . This looks like "unzipping of the DNA molecule " . However the single strand binding proteins "SSBPs" bind with the newly separated strands to prevent re-annealing of two strands (figure43) helixase and SSBPs . The helixase start to unzipping the DNA at Ori " origin of

replication " which is an (A-T) rich region in the DNA and it recognized by special enzyme system. In prokaryotes the separation is performed at single Ori , while in eukaryotes there are multiple ori (figure 44).

As two strands unwound and separated , they form a "V" shape where active synthesis occur . This V shape called the replication fork (figure 45).

The progression of replication fork result in more unwinding of the DNA this eventually stops the replication "**supercoiling**" (figure 46) . **Topoisomerases** prevent and correct this topological problem. They do that by binding to the DNA and cutting the backbone of one strand "topoisomerase 1" or both strands "topoisomerase 2"

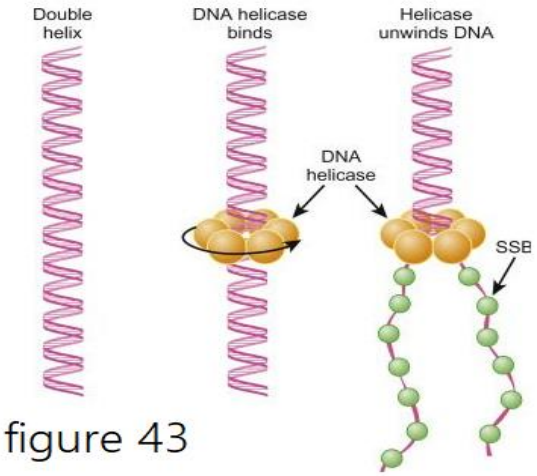
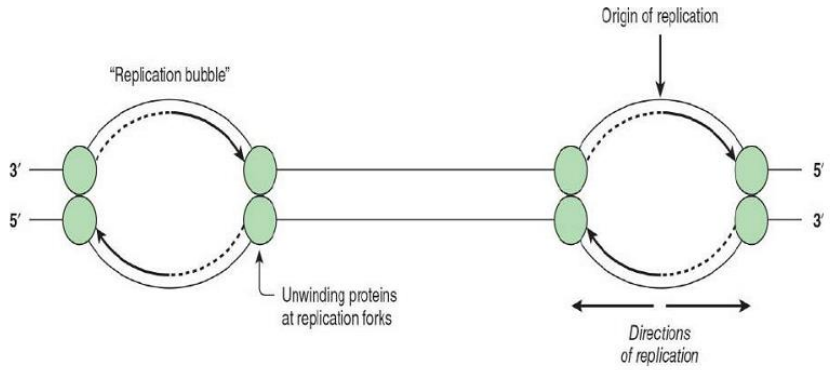


figure 43



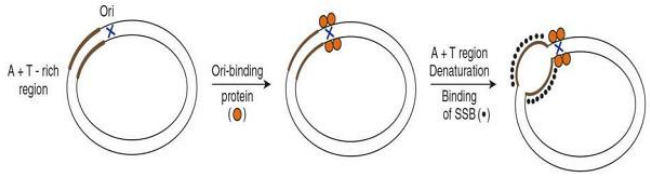


figure 44

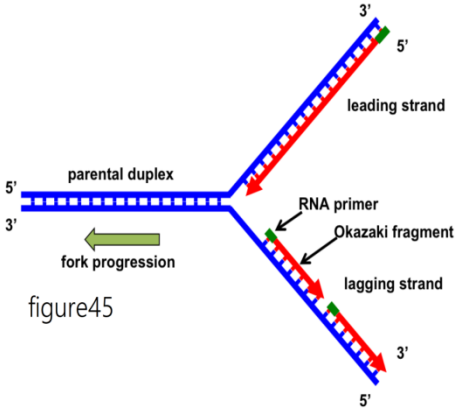


figure45

figure 46

2.RNA primer formation

The DNA polymerase which is the enzyme that responsible for synthesis of new strand as we will see next , require a "start point" represented as RNA primer(figure 47)

Synthesis of RNA primer needs **Primosome** , which is a complex of an Enzyme called **primase** " RNA polymerase in it nature" and **hilixase protein**. The primers are removed later in replication.

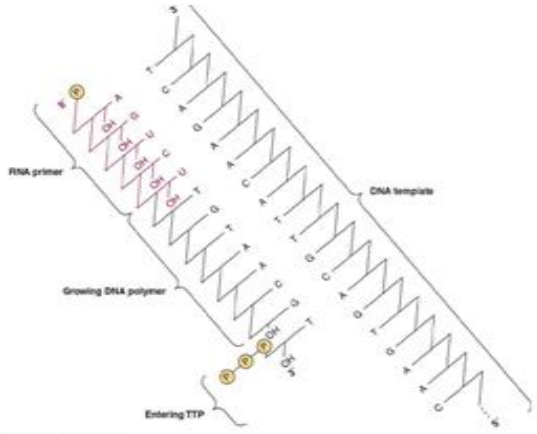
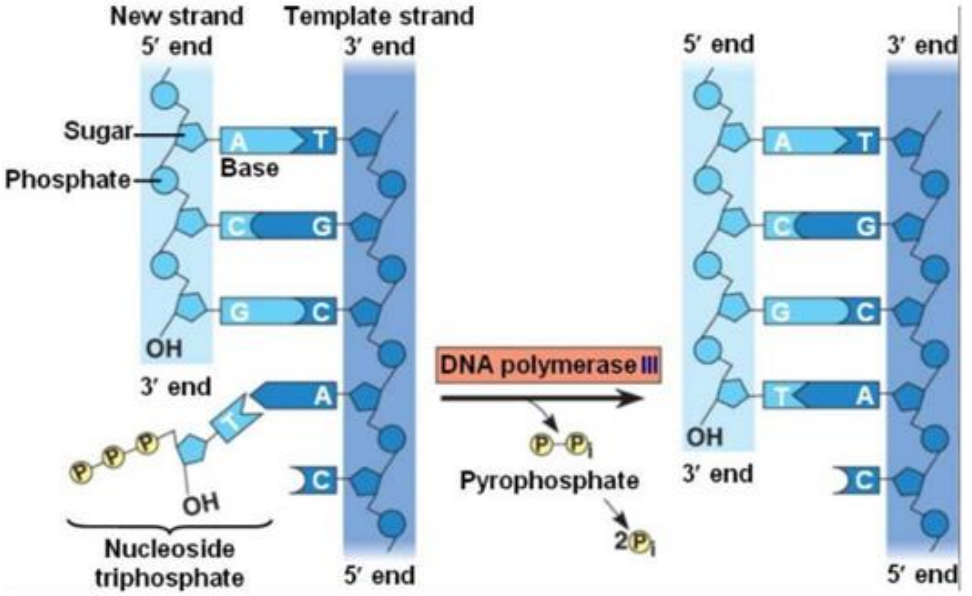


figure 47

3. Synthesis of new DNA strand

Enzymes that responsible for synthesis of the DNA are called DNA polymerases , also they proofread and involved in DNA repair .they synthesize the new chain by adding new deoxy ribonucleotides "one after another " at the **3` end** of growing polynucleotide chain , the addition of nucleotides depends on the base pairing rules "complementary to the old strand " , but this process not a free-errors , in fact mismatching errors talk place every replication cycle , some DNA polymerases proofread the newly synthesis strand others remove the miss-incorporated nucleotides and fill the gap of removed nucleotides as a part of DNA repair system "see DNA repair " . Note that some DNA polymerases can function as proofreader and repairer at same time . However in eukaryotes DNA polymerase,α , β

Gamma,delta and ebsilon are responsible for the replication "and also repair " while in prokaryotes DNA polymerase 1,2,and 3 . (Figure 48)
Remember that DNA pol **alpha** and **delta** "in eukaryotes" , and DNA polymerase **3** "in prokaryotes" are the major enzymes involved in DNA replication.



E coli	Mammalian	Function
I	α	Gap filling and synthesis of lagging strand
II	ε	DNA proofreading and repair
	β	DNA repair
	γ	Mitochondrial DNA synthesis
III	δ	Processive, leading strand synthesis

figure 48

Because DNA polymerase synthesize DNA strand from 5` to 3` only and There are two anti- parallel strands with different orientation in the DNA "that desired to be replicated " the two strands replicated differently i.e two different templates (figure49)
One of the strands oriented in 3`to 5` direction "towards the replication fork " here the synthesis is continuously ,thus it called called "leading strand" . The other strand orientated in 5`to3` direction "away from replication fork" here the synthesis is discontinuous "thus called lagging strand" and need alot of RNA primers "more than leading strand ". Every RNA primer linked to small segment of DNA "together they called okazaki fragment (figure 50)

4. **Removal of RNA primers**

The RNA primers are removed and gaps that formed are filled by DNA polymerase 1/alpha with the proper deoxy nucleotides "according to the base pairing rules" , then the DNA segments are sealed together by DNA ligase (figure 51)
(Figure 52 : funny picture shows the main enzymes and proteins that involved in replication)

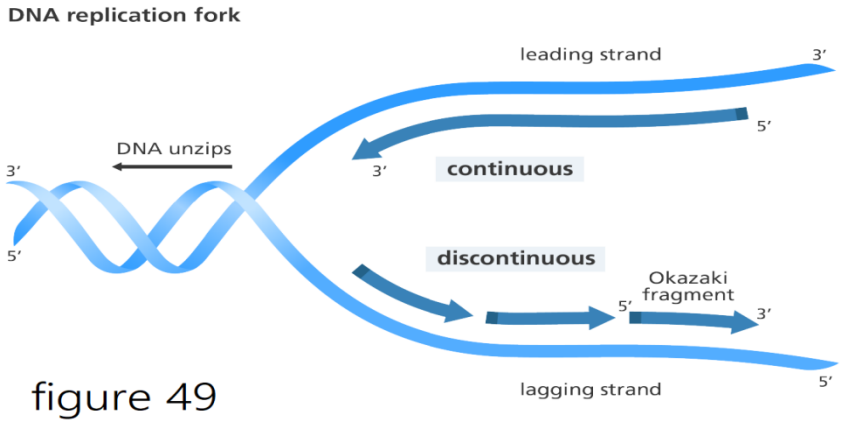


figure 49

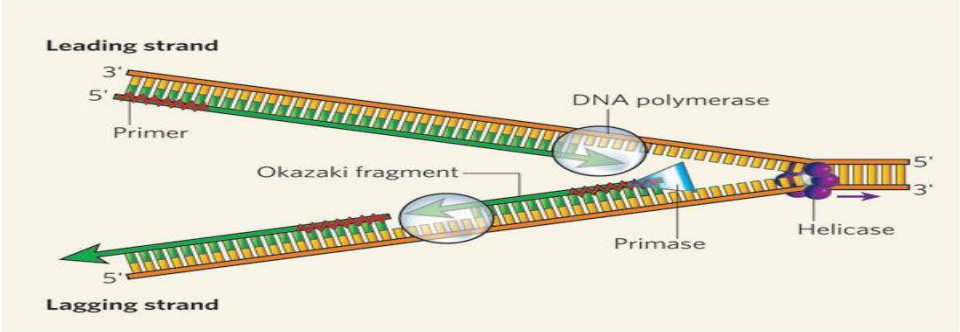


figure 50

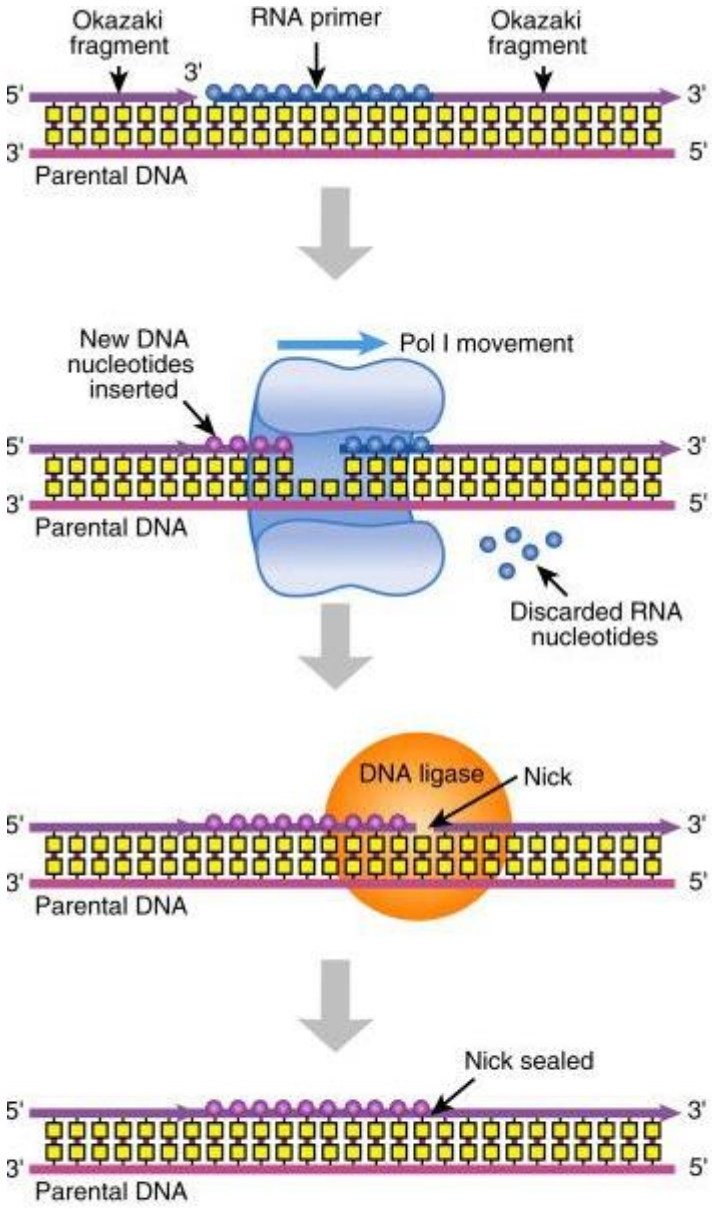

Okazaki fragment
RNA primer
Okazaki fragment
5'
3'
3'
5'
3'
5'
Parental DNA
New DNA nucleotides inserted
Pol I movement
5'
3'
3'
5'
Parental DNA
Discarded RNA nucleotides
DNA ligase
Nick
5'
3'
3'
5'
Parental DNA
Nick sealed
5'
3'
3'
5'
Parental DNA

figure 51

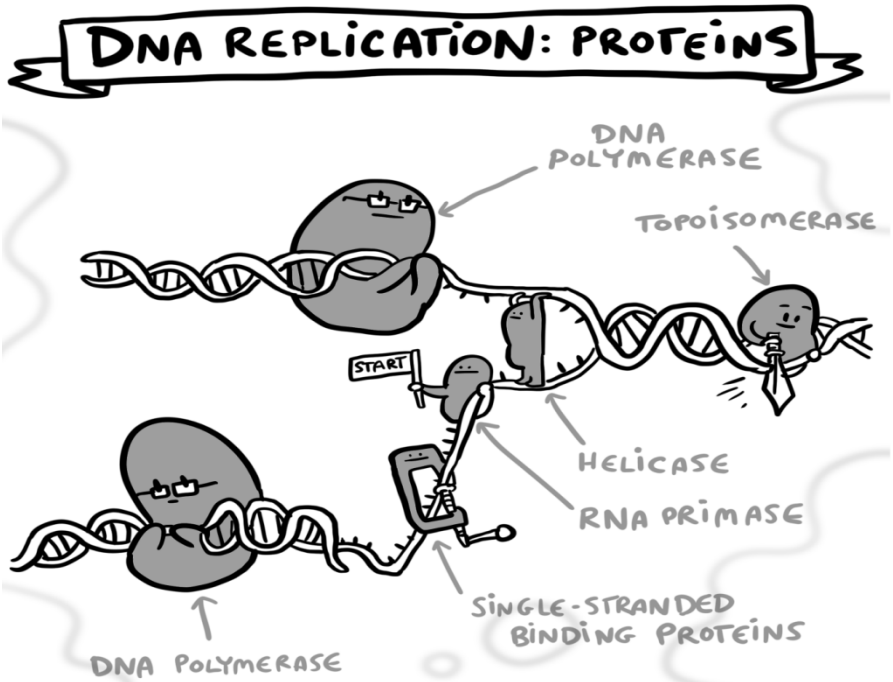


figure 52

Cell cycle

See cell cycle and cancer

Transcription

Transcription is the process of synthesis RNA from a DNA segment , this segment can be 1 encoded for protein , this transcribed into mRNA ,2 not encoded for protein and this transcribed into noncoding RNAs "ncRNAs" such as tRNA ,rRNA and others . Nearly all types of RNA are involved in protein synthesis in one way or another

Transcription proceeds in the following general events

1.RNA polymerase with one or more general transcription factor bind with specific sequence in DNA that represents the start line called **promoter** .

2.RNA pol separates the two strands of DNA by breaking the **hydrogen bonds** between the two strands " helixase- like action" , this lead to formation of "transcriptional bubble. Note that here there is no SSBPs thus once the RNA pol is move the two strands behind it will reanneal

3.RNA polymerase adds RNA nucleotides that complementary to those in The DNA segment according to the base pairing rules but here U instead T . RNA polymerase catalyze phosphodiester bonds formation between nucleotides to form RNA strand
4. Hydrogen bonds between DNA segment and newly synthesized RNA broken and this freeing the RNA strand
5. RNA molecule undergoes modifications according to his type

The steps of transcription are **initiation**, **elongation** and **termination**

A. Initiation

RNA polymerase

Prokaryotic cells such as bacterial cells contain one type of RNA pol responsible for synthesis of all types of RNAs . It consist of five subunits: two identical α, β , β' and omega subunits. In bacteria there is one general transcription factor known as sigma factor which guides RNA polymerase to the promoter site . The five subunits together are called RNA polymerase Core enzyme" , when sigma factor is bind to the core collectively are called RNA polymerase Hollow enzyme. (Figure 53) .Note that the core enzyme also called E

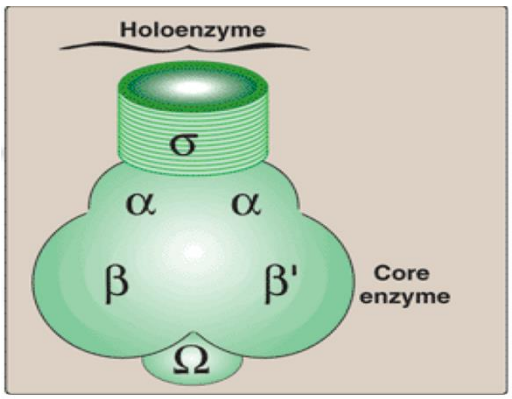


figure 53

Subunit	Gene	Function
α (alpha)	*rpo*A	Enzyme assembly
β (beta)	*rpo*B	Nucleotide binding
β' (beta prime)	*rpo*C	Template binding
σ (sigma)	*rpo*D	Promoter binding

Core: $\alpha_2 \beta \beta'$

Holoenzyme: $\alpha_2 \beta \beta' \sigma$

Eukaryotic cells contain three distinct types of RNA polymerase 1,2and 3 , their functions in (figure 54)

The three eukaryotic RNA pol are distinguished on the basis of their sensitivities to alpha amanitin which is a peptide toxin extracted from Amanita phalloides mushroom , and it inhibits "variable degrees " the RNA polymerases (Figure 55)

RNA polymerase in eukaryotes contain subunits homologous to each of the five subunits in bacteria, and also contain additional subunits. Eukaryotes also contain general transcription factors corresponding sigma factor in bacteria, for example the RNA polymerase 2 has six different general TFs: TF2A ,TF2B ,TF2D, TF2E ,TF2F and TF2H

Type of Polymerase	Responsible to Produce	Location
RNA Polymerase I	rRNA (18 s , 5.8s, 28s)	nucleolus
RNA Polymerase II	mRNA and small nuclear RNA	nucleoplasm
RNA Polymerase III	tRNA and 5s r RNA	nucleoplasm

figure 54

Eukaryotic RNA polymerases

Enzyme	Sensitivity to α-amanitin	Products
Poly (I)	Insensitive	rRNAs (28S, 18S, and 5.8S)
Poly (II)	Highly sensitive	mRNAs and snRNA
Poly (III)	Moderately sensitive	tRNA and 5SrRNA

55

figure 55

Promoter Region

The promoter Region is a short nucleotides sequence located upstream "just few base pairs" to the gene transcription start site "TSS" . It promote the gene recognition "and binding" by RNA polymerase.
A Promoter Region usually **doesn't vary too much** from one organism to another .

Prokaryotic promotors

-**Pribnow box** which is a stretch of six nucleotides (5` TATAAT 3`) located about 10 bases upstream "-10" to the transcription start site .
Another one is **(5` TGTTGACA 3`)** which is located about 35 bases upstream "-35" (Figure 56)

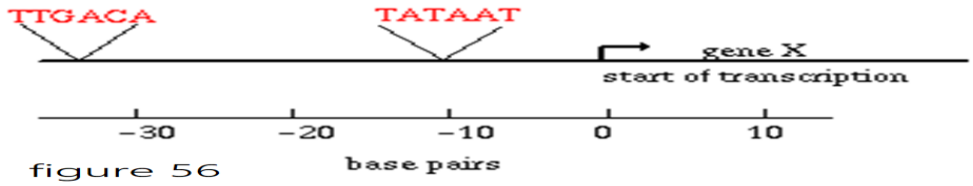


figure 56

Eukaryotic promotors

TATA box " **hognes box**" , corosponds pribnow box "in prokaryotes" , located about 25-35 base upstream to TSS.
CAAT "**Cat" box** , located about 70-80 bases upstream to TSS with sequence (5` GGC**CAAT**CT 3`) (Figure 57)

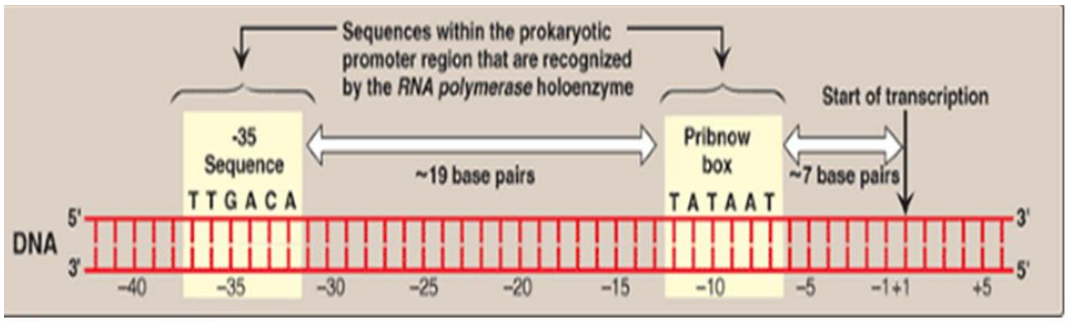


figure 57

Note that a specific sections of DNA known as enhancer sequences , there are not promoter sequences , the enhancer sequence basically is the sequence in which a specific regulatory protein can bind to , and once that protein binds to the enhancer region it promotes the process of transcription. Enhancer can be found about several thousands nucleotides upstream or downstream ,and it is not found in prokaryotes .

B.Elongation

Transcriptional elongation is the process in which RNA polymerase actually synthesize the RNA molecule. Once the RNA polymerase unwind the DNA it creates a "bubble" called transcription bubble (figure 58)
Note that only one of two strands is actually used to synthesizing the RNA molecule , thus strand called template strand or antisense strand , the other strand called the -

-Codding strand or sense strand which is the strand that actually being copied i.e nucleotides in the Codding strand are identical to those in corresponding RNA molecule but the only difference is that the **T** replaced by **U** (figure 59) .
Like DNA polymerase the RNA polymerase can only read the strand from 3`to 5` , and synthesize the RNA strand begins at 5` end and ends at the 3` end (figure 59) .
RNA polymerase adds the nucleotides to the 3` end of the growing RNA chain according to the base pairing rule with taking account for "T replaced by U ".
Multiple RNA polymerase enzymes can act on a single gene at the same time

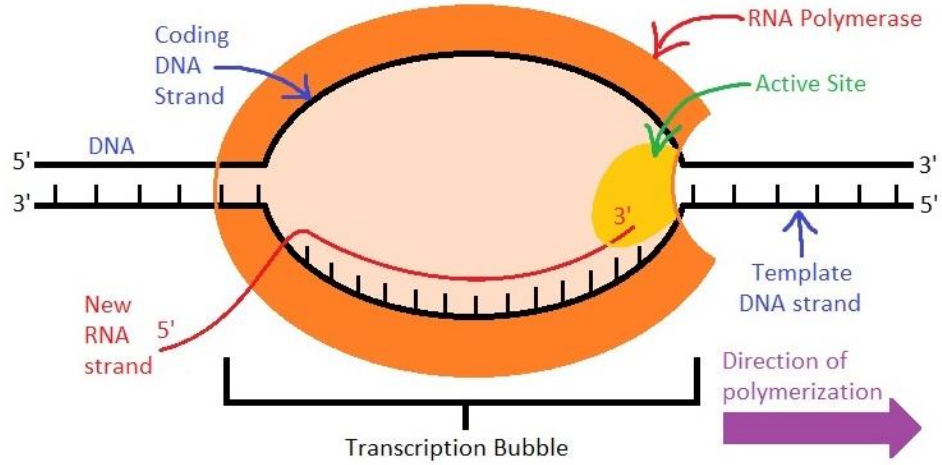


figure 58

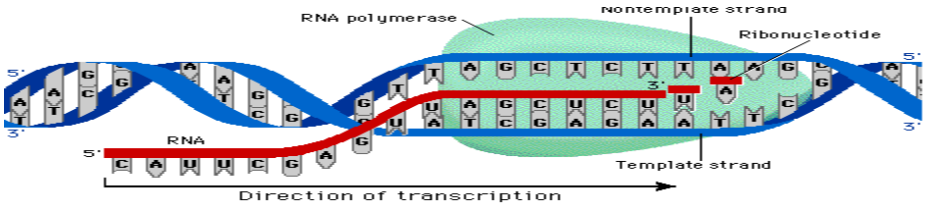


figure 59

C. Termination

at the end of transcribed DNA segment We have termination sequence which is a stretch of DNA that carries the signal for terminates the transcription.
In prokaryotes, the termination region can be recognized by either :
1 **Rho factor** : which required for release RNA pol and RNA strand from the DNA (figure 60 a)
2 **independently on rho factor :** RNA polymerase continue in transcription until it reaches the termination sequence and transcribed it , termination sequence in this case encoded for a signal called "hair pen" (figure 60 b)

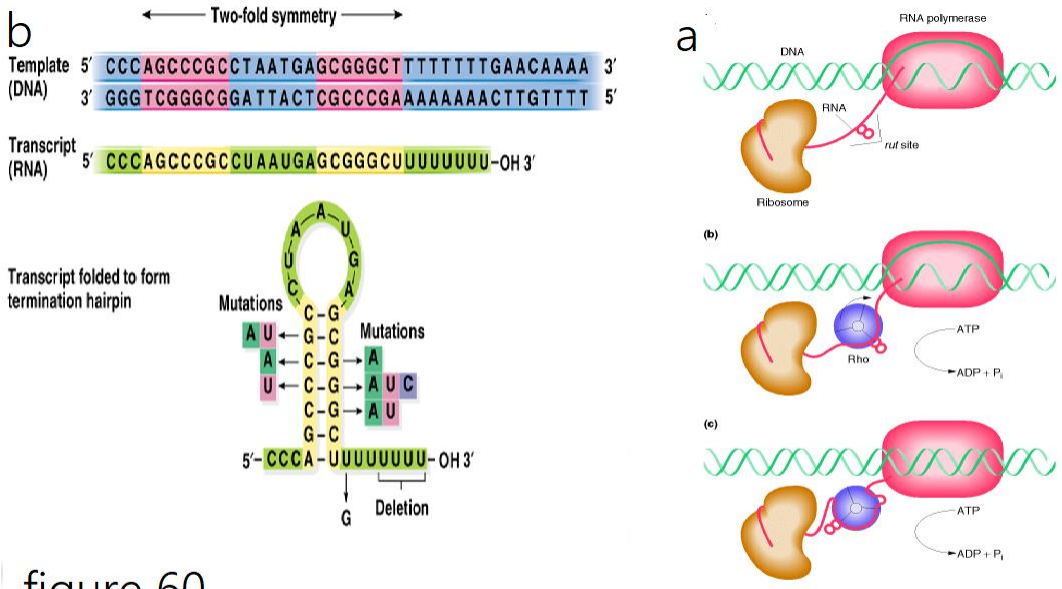
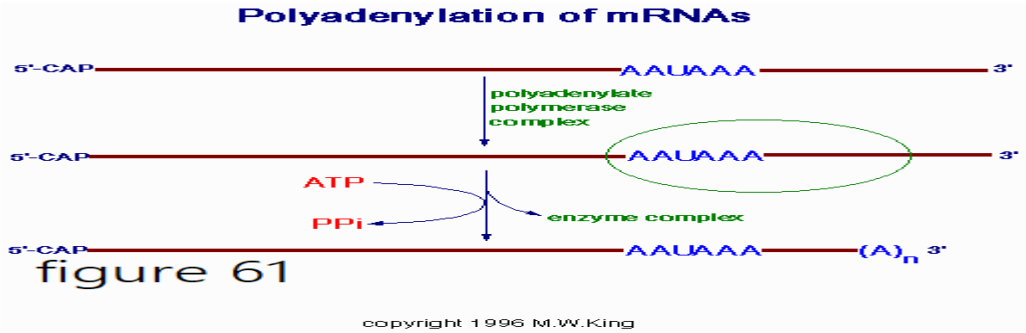


figure 60

In Eukaryotes the termination process is less known, specific termination factors like sigma factor in prokaryotes may involved , however special **poly adenylation sequence** (AAUAAA) found nearly to the 3`end of mRNA ,and it serve as cleavage signal (figure 61)



Notes

The raw product of transcription "primary transcript" is called heteronuclear RNA .
In Eukaryotes the primary transcript "heteronuclear RNA " undergoes processing called Post transcription modifications.
Transcriptional unit is stretch of DNA that include signals for transcription initiation, elongation and termination.
Like DNA , during nucleotides"tri phosphate form" incorporation into the growing chain the , the loss of pyrophosphate occur " β and gamma phosphates"

Post transcriptional modifications

In **Eukaryotic cells** , RNA is synthesized but must be processed into a mature one to be functioning. This processing called post transcriptional modifications. Note that both coding and noncoding RNAs reported undergo modifications

Modifications on mRNA

A. Addition of Cap structure, a 7methyguanosine "unusual modified base "Cap is added to the 5`end of nascent mRNA (figure62). The 7-methylguanosine tri-phosphate linked to first nucleotide at **5`end** of the nascent mRNA by unusual **5`-5` triphosphate linkage** .the cap function is to protecting the mRNA from exonucleases and help the ribosomal attachment to the mRNA thus help the translation

B. 3`-OH polyadenylation , 20-250 adenosine nucleotides added at the 3` end of the nascent mRNA (figure62) , this catalyzed by enzyme called polyadenylate polymerase. Poly A tail prevents the mRNA from exonucleases . Poly A tail is not required for translation, but it affects the stability of transcript in cytoplasm. As mRNA stay in cytoplasm the long poly A tail is gradually removed. Once poly A tail is gone, the mRNA will soon be destroyed.

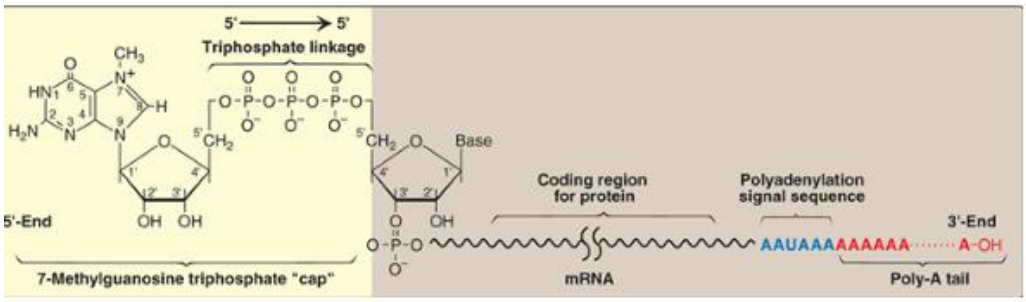
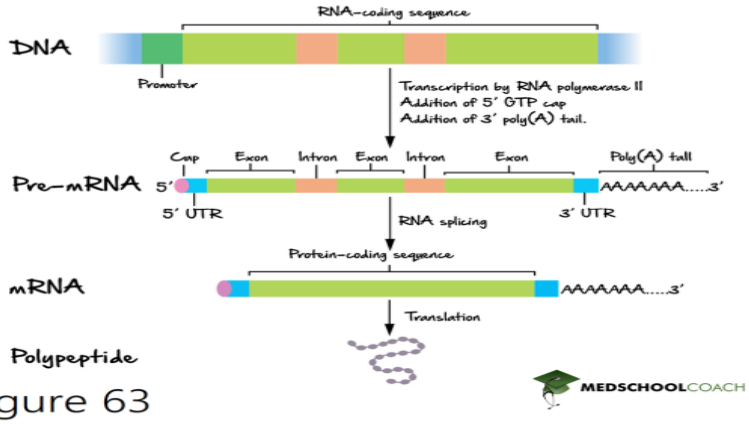


figure62

C. Splicing

Eukaryotic genes contain inside themselves a non- coding protein areas called introns, while the coding areas called exons (figure 63), however the introns must to be removed . The process of removing introns and reconnecting exons is called splicing .introns are removed and degraded while pre mRNA is still in nucleus . The splicing process is done by protein complexes called splicosomes , which are composed from proteins and RNA molecules called Small nuclear RNAs "snRNA " .
It is not necessarily to know about the mechanism of splicing see figure 64 splicing



fgure 63

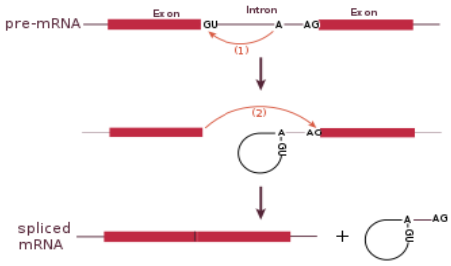


figure 64

Alternative Splicing

In alternative RNA splicing, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns (Figure 65)

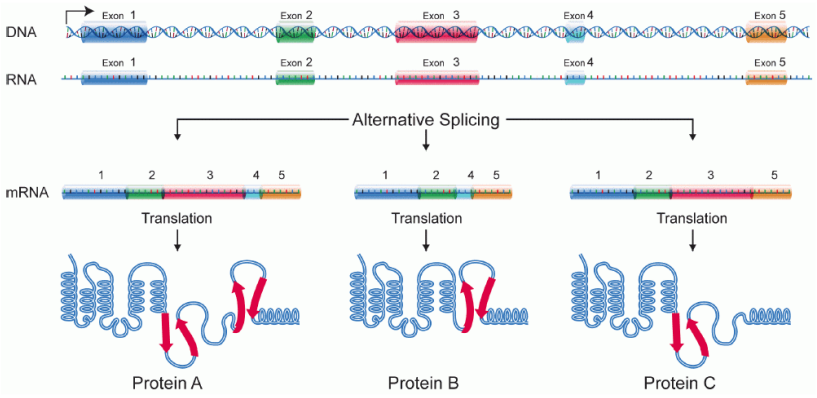


figure 65

D. RNA editing

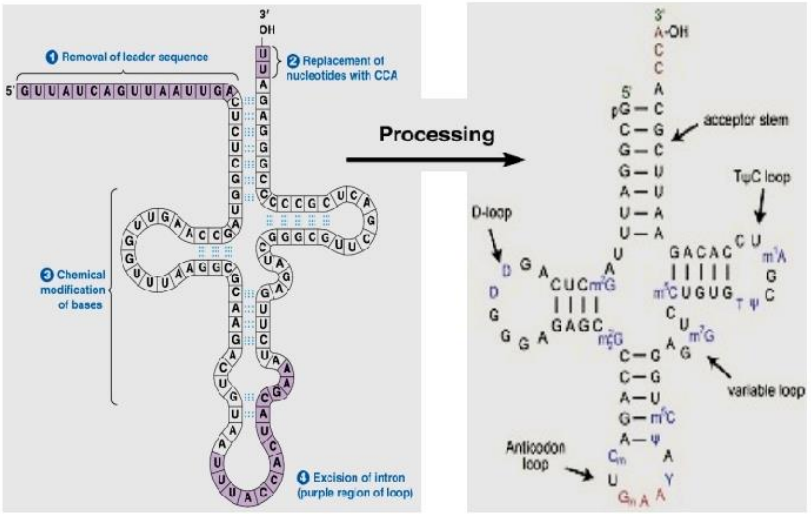
RNA editing can be considered as co or post transcriptional modification . Some mRNAs are altered to contain bases not encoded in genome , to be more comprehensive the sequence of the mRNA can undergoes alterations, for example ApoB gene and it mRNA , ApoB100 and ApoB48 are encoded by the same gene , why in the intestine the ApoB gene gives a protein with molecule weight 48kd , while in liver gives 100Kd protein ? The ApoB gene and subsequently mRNA contains the sequence CAA nearly in the middle of mRNA molecule, in the intestine the enzyme cytidine deaminase converts this sequence to **UAA** , the UAA is one of the stop codons which are termination signals in translation "protein synthesis processes "

Remember that the mRNA in eukaryotes is monocistronic "one mRNA carry instructions only for one polypeptide " , while in prokaryotes mRNA can be polycistronic .

Modifications on tRNA :

As shown in (Figure 66)

-Replacement of nucleotides at 3` end with CCA
-Chemical modifications of some nitrogenous bases like pseudo uracil ,ribo thymine "which they are found in various palaces specially in TuC loop" and dihydrouracil " in the D loop"
-Excision of introns are also occur "here the term introns and exons are not related to protein coding but functional RNA coding "



fiure 66

Modifications on rRNA

In Eukaryotes there are four types of rRNA , three of them "18s, 5.8s and 28s" are made by chemical modifications and cleavage of a single precursor unit called "45s pre rRNA "which is transcribed by RNA pol1 (Figured 67)
Non coding regions between the transcription units are removed to convert the precursor molecule 45s pre rRNA to mature 18s , 5.8s and 28s rRNAs . In addition to this cleavage, rRNA processing involves addition of methyl to the bases and sugars of specific nucleotides

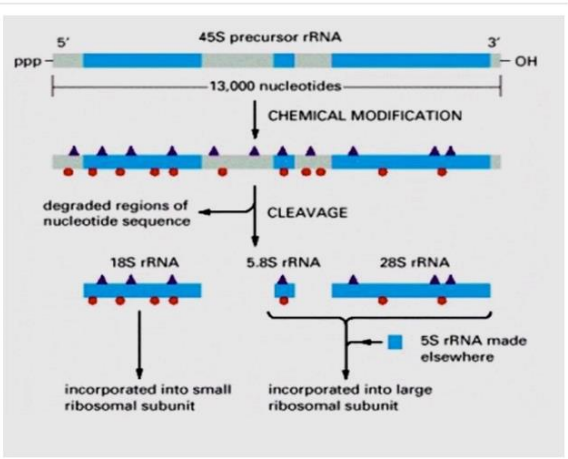


figure 67

Notes :
The table in the (figure 68) shows the structure and function of mature RNAs "major three"

	mRNA	rRNA	tRNA
What does the letter stand for?	Messenger RNA	Ribosomal RNA	Transfer DNA
Function	Takes information from DNA to ribosomes	Where proteins are made	Delivers amino acids to ribosome. Anticodon matches the codon on mRNA strand
Image			

68

Reverse transcriptase

Some viruses (such as HIV, the cause of AIDS), have the ability to re-transcribe RNA into DNA. HIV has an RNA genome that is reverse transcribed into DNA. The resulting DNA can be merged with the DNA genome of the host cell. The main enzyme -

- responsible for synthesis of DNA from an RNA template is called **reverse transcriptase.**

In the case of HIV, reverse transcriptase is responsible for synthesizing a complementary DNA strand (cDNA) to the viral RNA genome. The enzyme **ribonuclease H** then digests the RNA strand, and reverse transcriptase synthesize a complementary strand of DNA to form a double helix DNA structure ("cDNA"). The cDNA is integrated into the host cell's genome by the enzyme **integrase** , which causes the host cell to generate viral proteins that reassemble into new viral particles.

Some eukaryotic cells contain an enzyme with reverse transcription activity called **telomerase**. Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes. Telomerase carries an RNA template from which it synthesizes a repeating sequence of DNA, or "junk DNA". This repeated sequence of DNA is called a telomere and can be thought of as a "cap" for a chromosome. It is important because every time a linear chromosome is duplicated, it is shortened. With this "junk" DNA or "cap" at the ends of chromosomes, the shortening eliminates some of the non-essential, repeated sequence rather than the protein-encoding DNA sequence, that is farther away from the chromosome end.

Telomerase is often activated in cancer cells to enable cancer cells to duplicate their genomes indefinitely without losing important protein-coding DNA sequence. Activation of telomerase could be part of the process that allows cancer cells to become immortal. The immortalizing factor of cancer via telomere lengthening due to telomerase has been proven to occur in 90% of all carcinogenic tumors in vivo with the remaining 10% using an alternative telomere maintenance route called ALT or Alternative Lengthening of Telomeres.

Transcription ihibitors

Actinomycin D bind to the DNA and prevent movement of RNA polymerase enzyme and thus inhibit gene transcription .

Genetics code and translation

The central dogma of molecular biology tells us that the flow of genetic information in all organisms " the retroviruses are exception " is from DNA to RNA to protein (figure 69). The process of coping information from DNA to form RNA is called transcription, and the process of synthesizing protein from RNA is called translation. Its not difficult to imagine how transcription occurs because both DNA and RNA use the same language, they consist of the same building blocks . But RNA and protein are use two different languages i.e RNA use the nucleotide language and protein use amino acid language . Thus the process of translating the nucleotides sequence to amino acids sequence in protein is called translation .

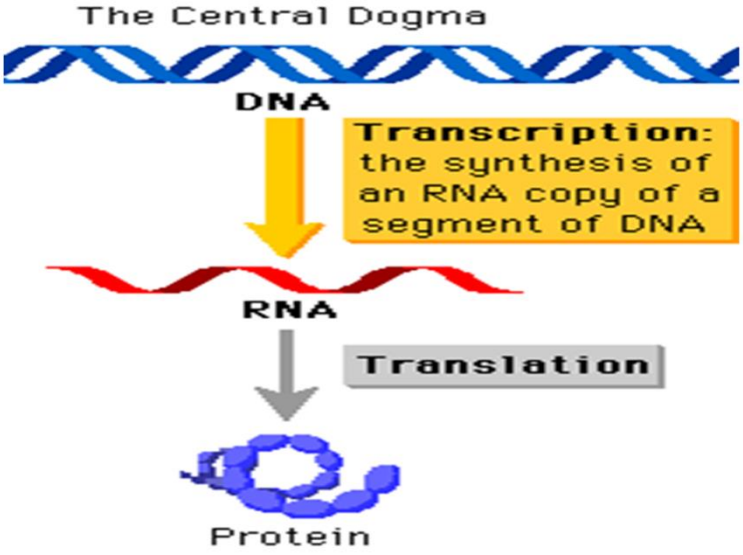
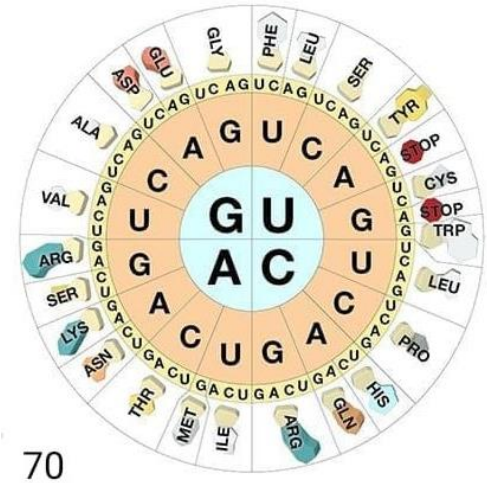


figure 69

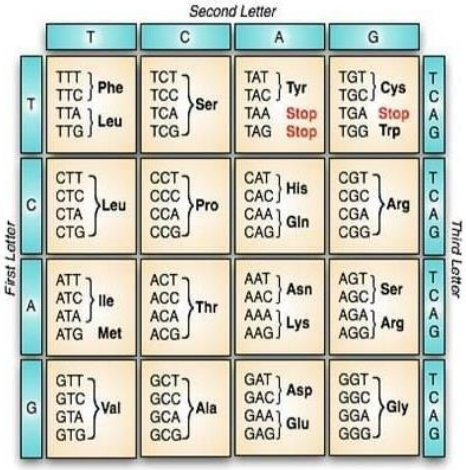
The genetic code

Genetic code is the system that is used by the cell "ribosomes" to translate the language used by the RNA "nucleotides sequence " to the language that used by protein "amino acid sequence " . In simple genetic code is the links between sequence of nucleotides in mRNA and sequence of amino acids in protein .it consists of codons , the codon is a sequence of three nucleotides .each codon is corresponds specific amino acid , for example CGC is encoded for Arginine amino acid .

There are four types of nucleotide in mRNA are used to produce three nucleotide codons , thus we have 64 codons (4×4×4) (Figure 70) ,three of them are stop codons to terminate the translation process " UAA, UAG and UGA) , the rest are encoded for amino acids .....but we have only 20 amino acids enter in the structure of protein , how this occurs? 61 codons corresponds 20 amino acids !? And a single codon corresponds a single specific amino acid, how this occurs? The answer is a one amino acid can have more than one codon corresponds it .



70



Characteristics of the genetic code :

-unambiguous: each specific codon always encoded for specific amino acid

-degeneracy "or redundancy " : multiple codons can encoded for single amino acid

-non overlapping: the codons don't overlap each others that is means a particular Nucleotide in mRNA is not used in two different codons, the best way to understand this is seeing (figure 71)

There is no punctuations : no punctuation between codons i.e there is no boundaries In between codons (figure 72)

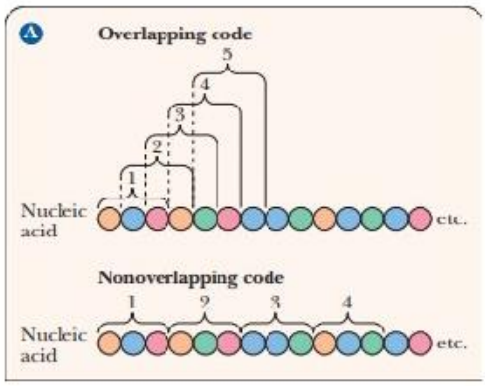


figure 71

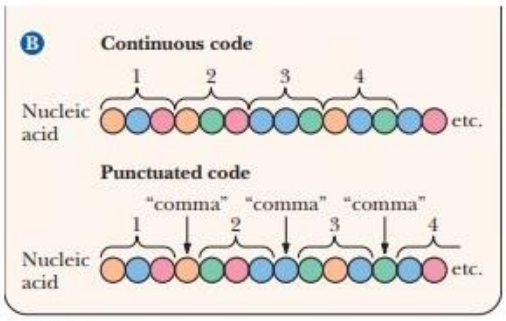


figure 72

Universality: with neglecting the exceptions" few codons in prokaryotes and eukaryotic mitochondria " , the genetic code is the same in all organisms i.e the X codon is encoded for Y amino acid in all organisms. Few codons in prokaryotes and eukaryotic mitochondria are exceptions for example AGA is encoded for Arginine amino acid but in mitochondria is a stop codon .

Note

The start codon "also called the initiation codon" that carries signal for initiating translation is AUG and it encoded for methionine amino acid , but that dosent mean the first amino acid in all proteins to be methionine amino acid because proteins undergo a post translational modifications PTMs .

Translation

Translation is the production of a protein by decoding a mRNA
Before discussing the translation, We should talk about tRNA and ribosome

tRNA

Small single stranded RNA molecule, about 70-95 nucleotides, in addition to A,G,C and U tRNA contains modified nucleotides produced by chemical modifications of the bases of the four primary nucleotides
Each t RNA molecule is a cloverleaf structure , which is looks like a "L" shape in the three dimensional structure. They are three nucleotide sequence in tRNA represent the "anticodon" that complements the codons in the mRNA , the amino acids are carried on 3`end of tRNA on the OH of A . (figure 73)
We have 61 codons "without calculating the stop codons " ...are there tRNA corresponds to each codon i.e are there 61 tRNAs ? No they actually about 40 tRNAs ...how this occurs? In fact in some tRNAs a single anticodon can recognize several codons "on mRNA" , because the 3rd base in the codon "or 1st base in the anticodon" is able to base pairs less specifically and doesn't obey the base pairing rules. The explanation of this phenomenon was proposed by Francis crick as wobble hypothesis (figure 74) .
The wobble at the 3rd base of codon in mRNA " correspond to 1st base of anti codon in tRNA " is one of the characteristics of genetic code

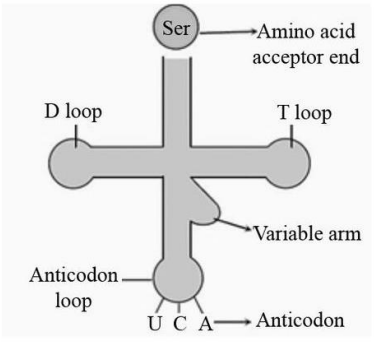


figure 73

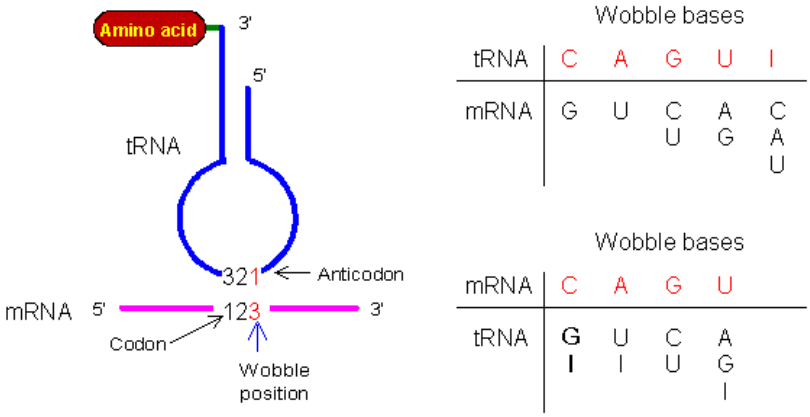


figure 74

Ribosomes

The ribosome composed of two subunits, large subunit and small subunit . In prokaryotes the large sub unit is "50 s" which contains one rRNA with ~31 proteins , and small subunit is "30s" which contains two rRNAs with 21 proteins

While in eukaryotes the large subunit is "60s' which contains three rRNAs and 45 protein , and small subunit is "40" which contains one rRNA with 33 proteins.

The two ribosome subunits have irregular shapes which fit together in such a way that cleft informed through which mRNA passes as ribosome move along it during translation(figure 75)

50s and 30s "prokaryotes " are combined together to form 70s

60s and 40s " eukaryotes" are combined together to form 80s

What exactly S means? The s" strand for sved-berg unit which is a unit that measures the rate at which the given particle "ribosome subunits in this case" sediment. Svedberg unit doesn't summated mathematically

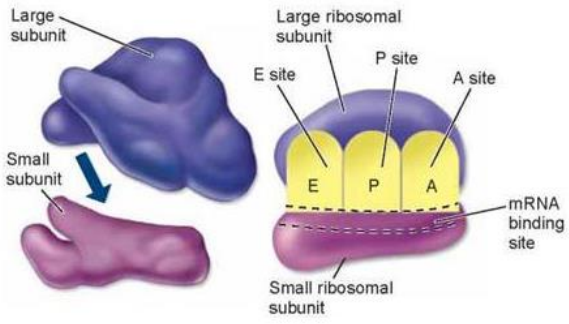


figure 75

Translation proceeds in four phases

0- activation of amino acids

1- initiation

2-elongation

3- termination

Activation of amino acids

In the activation of the amino acids, the correct amino acid is is covalently linked to the correct tRNA . In fact these step technically is not a step in translation, but it is required for translation process . The amino acid is joined by its caroxyl group to 3` OH of the tRNA " OH group of the A in CCA sequence at the 3`end " by ester linkage . When the tRNA has a linked amino acid it called "charged tRNA"

The enzyme aminoacyl.tRNA synthestase catalyze the attachment of tRNA molecule to its respective amino acid . There are at least one amino acyl tRNA synthetase for each amino acid (Figure 76)

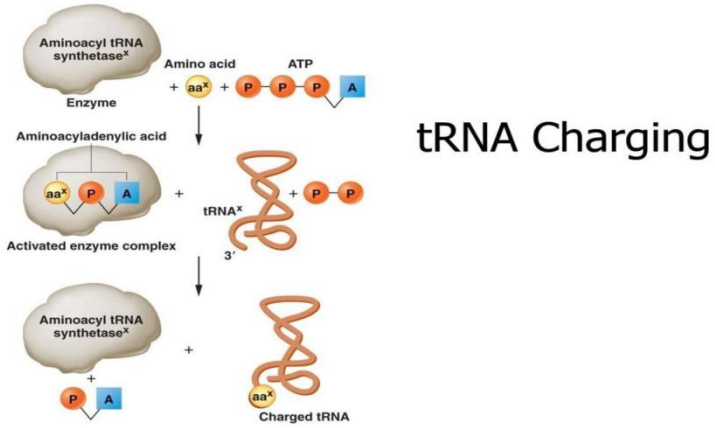


figure 67

Initiation

The initiation in both prokaryotes and eukaryotes require a special proteins known as initiation factors "IFs" in prokaryotes, and eukaryotic initiation factors "eIFs" in eukaryotes .

In **Eukaryotes** a specific initiator **tRNA-methionine** required to incorporates the initial methionine residue in all proteins ", It is not necessary for methionine to be the first amino acid in all proteins because proteins are subject to PTMs . In prokaryotes a specific version of tRNA-tRNA is required for initiation " **formylated methionine** instead the methionine " (Figure 77)

There are some differences between the initiation in prokaryotes and initiation in eukaryotes but the general idea is same , small and large ribosomal subunits assembled on the start codon near the 5`end of the mRNA by helping of many protein factors (IFs/eIFs) .

In Eukaryotes , 60s and 40s of ribosome are found in their dissociated state , eIF-1 and eIF-3 are bind to the 40s subunit favoring anti-association of 60s . In other place met-tRNA bind to a **binary** complex which is complex formed by eIF2 and GTP to form a **ternary** complex " met-tRNA , GTP and eIF2" . Then the ternary complex bind to 40s to form the **43 pre initiation complex** . Then mRNA is guided by "eIF4" to 43s pre- initiation complex to form the "**48s initiation complex** in process coupled with ATP hydrolysis (Figure 78)

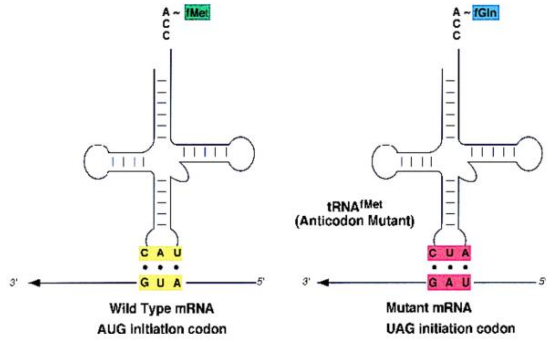


figure 77

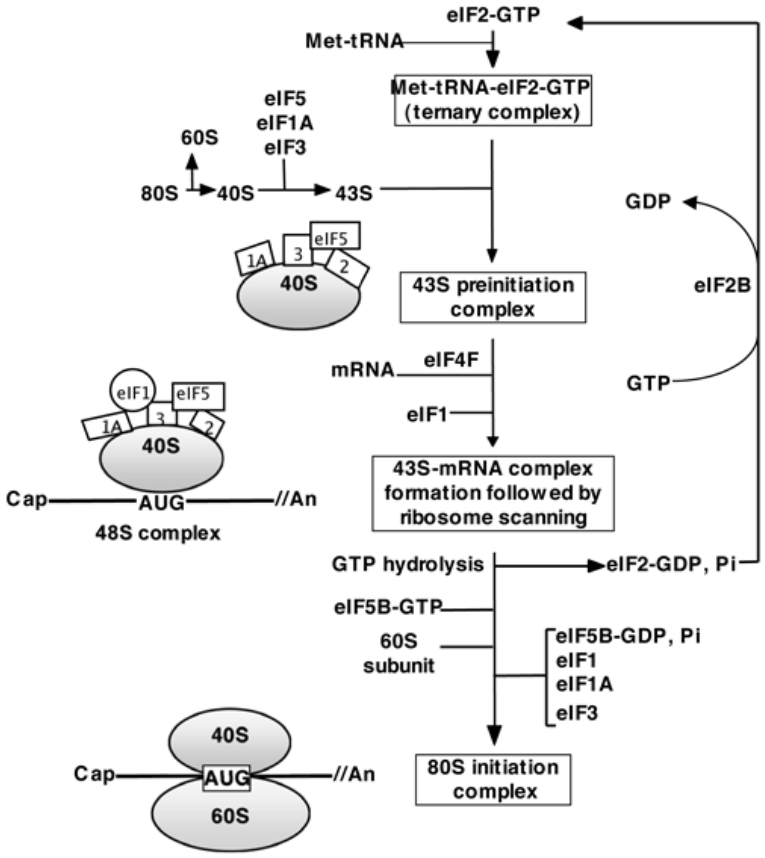


figure 78

Elongation

like initiation, elongation require specific protein factors known as elongation factors "in prokaryotes "Efs" / Eukaryotic elongation factors "eEFs" in eukaryotes . We will discuss the process in eukaryotes only .

The elongation of polypeptide chain involves the addition of amino acids to the carboxyl end of the growing polypeptide chain . During elongation the ribosome move on the mRNA from **5` to 3`** end .

In the step of elongation, the second amino acid "amino acid-tRNA" of the polypeptide is carried to the A site of the ribosome by the "eEF1-GTP complex" . Remember that the tRNA anticodon bind with mRNA codon . Now the P site occupied by met-tRNA and the A site occupied by the second amino acid-tRNA with eEF-1 and GTP , however the GTP will hydrolysed and eEF-1 departs .
Then by the catalytic activity of the 28s rRNA a peptide bond formed between the second amino acid and Methionine, result in di-peptidyl-tRNA in the A site , this called transpeptidation" .
To add a new amino acid to the growing chain, the A site must be freed in order to accept a new amino-tRNA .
The final step in elongation is translocation which is the process of moving peptedyl-tRNA from The A site to the P site , in fact this result from the ribosmal moving on the mRNA from the 5` to 3` . The moving or" translocation" requires translocase enzyme, eEF2 and GTP hydrolysis. Now after moving of the the old uncharged-tRNA is located in the exist site "E site" , pipeptedyl-tRNA in the P site and the A site is free and ready to accept a new amino acid-tRNA . The same scenario repeated until reaching a stop codon . (Figure 79)

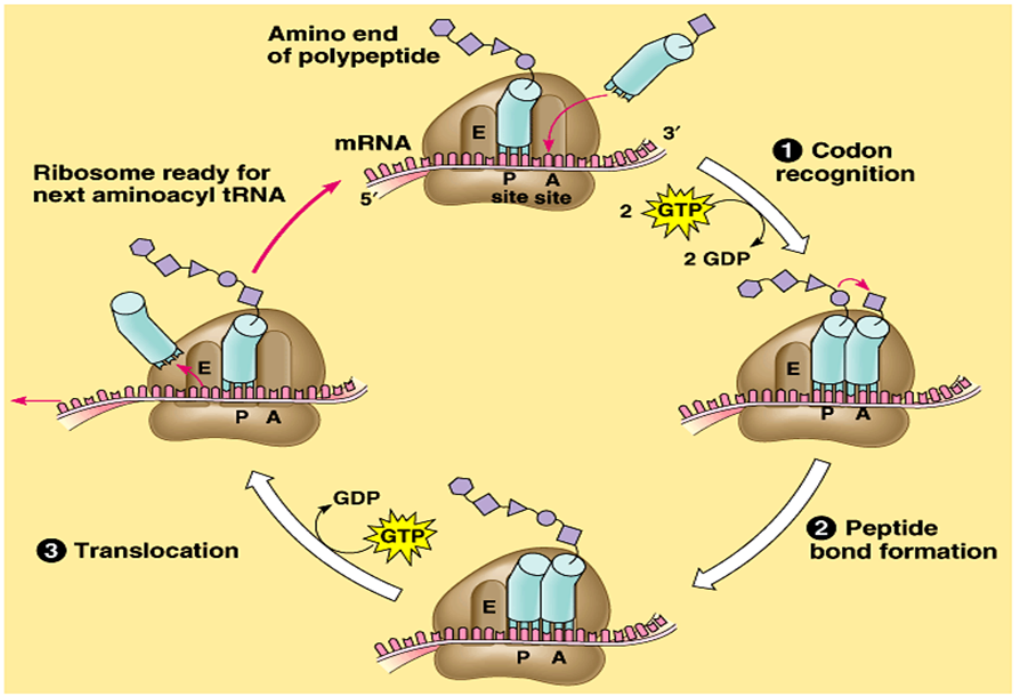


figure 79

Termination

Like initiation and elongation , the termination requires special protein factor(s) known as releasing factors "RF" in prokaryotes and Eukaryotic releasing factor "eRF" in eukaryotes .
The termination of polypeptide synthesis is happened the A site of ribosome faces a stop codon "UAA, UAG or UGA" when this occurs , no tRNA can recognize the stop codon but the releasing factor (RF / eRFs) recognize the nonsense stop codon and cause releasing of polypeptide chain .

Prokaryotes have three RFs while eukaryotes have only one eRF . In prokaryotes UAA and UAG are recognized by RF-1 , whereas RF-2 recognize UAA and UGA
Either RF-1 or RF-2 binds at the termination codon and induce peptedyl transferase to transfer the polypeptide chain to water molecule rather than to another amino acid-tRNA . There is also RF-3 but its function unknown.
Finally the dissociation of ribosome and releasing of mRNA occur
In Eukaryotes only one eRF recognize all stop codons and catalyze transferring of the polypeptide chain to water molecule. Finally ribosome will separated into large and small subunits and mRNA is released (Figure 80)

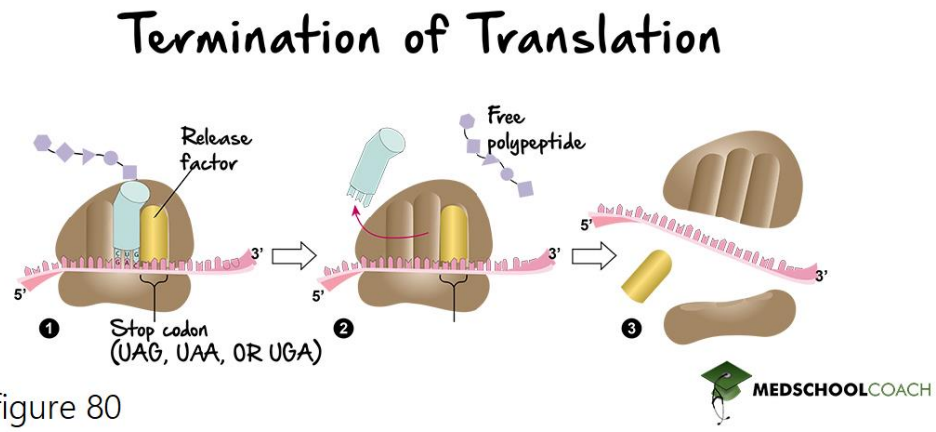


figure 80

Note

Polysomes

A single mRNA molecule can translated simultaneously by many ribosomes , spaced closely together .
Such a complex of one mRNA and number of ribosomes called polyribosome or polysome .
The simultaneous translation (figure 81) of a single mRNA by many ribosomes allows highly efficient use of mRNA .

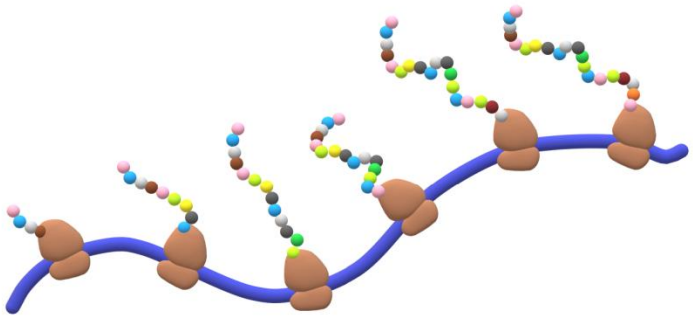


figure 81

Translation inhibitors

Many antibiotics such as Tetracyclines, lincomycin, erythromycin, streptomycin & chloramphenicol inhibit translation in prokaryotic ribosome (70s), and do not interact with eukaryotic ribosomes(80s) ,so they are not toxic to human.

-sreptomycin bind to the 30s subunit and interfering with protein synthesis.

-Tetracycline prevents the binding of aminoacyl-tRNAs to the A site.

-Clindamycin and erythromycin inhibit translocation.

-Puromycin is analog of tyrosinyl-tRNA , causes premature release of the polypeptide .

-Cycloheximide inhibits peptidyltransferase in the 60S ribosomal subunit in eukaryotes.

-Puromycin is analog of tyrosinyl-tRNA , causes premature release of the polypeptide .

- Cycloheximide inhibits peptidyltransferase in the 60S ribosomal subunit in eukaryotes.

-Diphtheria toxin: exotoxin of Corynebacterium diphtheriae ,specifically inhibits mammalian(eEF2) thus inhibit translocation.

-Ricin: toxic molecule ,from castor bean, inactivates eukaryotic 28S ribosomal RNA

Post translational modifications "PTMs"

PTMs refer to chemical changes that occur after protein has been translated .

Major PTMs :

A- phosphorylation

Phosphorylation is the addition of phosphate group into amino acid residue" usually serine or therionine " of the protein. The enzyme that catalyze the addition of phosphate to protein is known as protein kinase . The addition of the phosphate increase the hydrophilic characters of the protein . However the biological significance of this type of modification is seen in the cell cycle regulation "see cell cycle & cancer " and signal transduction "see hormones"

B.Methylation

Addition of methyl group onto certain amino acids via enzyme called methyl transferase increase the hydrophobic characters of the protein. The methylation os important in the epigenetic control of gene expression "see gene regulation "

C.Glycosylation

Protein glycosylation is the addition of sugar component onto protein, the addition of sugar is affects the protein folding process as will as changes the configuration, functional stability and distribution of this protein . One example of proteins that glycosylate are those proteins lies on plasma membrane and acting as receptors

D.Protyolysis

Protyolysis is the process by which certain types of enzymes known as proteases cut the protein . Some proteins are synthesized in the inactive form such as zymogens and pre-hormones , to activate this proteins the proteases must cleaves certain peptide bonds in those inactive proteins

E.N-Acetylation

Addition of acetyl group onto specific amino acids in protein is not only take place after translation ,but also during the translation too . Acetylation is important in regulation of gene expression.

F.Lipidation :

Addition of lipid component onto a protein us used to increase the affinity of membranes to this protein "because the membranes are composed mainly of lipids"

Mutations

Any sort of change or alteration in the DNA sequence -other than genetic recombination- is known as mutation .

Mutations are the source of the altered versions of genes . That is provide the raw material for evolution. However most mutations have no effect on the organism especially among the eukaryotes because huge portion of DNA "about 98%" is not protein coded i.e mutation in non coding DNA not result in an abnormal protein thus there is no effect on the organism.in the cases in which the mutation occurs without altering the normal functions of the mutated cells this termed a silent mutation

Genetic mutation can arise as result of two things 1 spontaneously: due to mistakes that take place in natural process inside the cell such as replication mistakes, 2 or can be induced , variety of physical and chemical "exogenous and endogenous " agents can lead to mutations such as mutagenic chemicals , UV rays ....etc

Types of mutations

There are two main types of mutations , point mutations and frame shift mutations

1 points mutation " base pair mutation or base pair substitution "

In this type the change occurs in a one base (figure 82), this can occur either in the coding or noncoding DNA. Sometimes a point mutation can occurs in the coding regions without alteration in the structure "and subsequently function" of the protein products ,Other times a point mutation can be incompatible with life as we will see

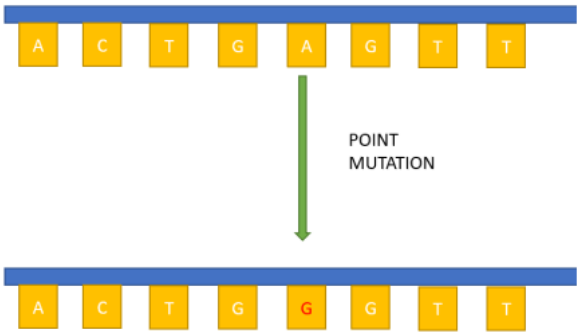


figure 82

The point mutation can be :

Transition, when one purine replaced by another purine i.e G->A , or one pyrimidine replaced by another pyrimidine i.e C->T

Transversion , when a purine replaced by pyrimidine or pyrimidine replaced by purine.

Point mutations show variable degrees of functionality

- imagine that , in the gene "X" , the codon CAA "glutamate" is mutated and become CAG "l glutamate" , the codon still codes the same amino acid , this is a silent mutation "no effect on function" .
- in the same "X" gene , the CAA "glutamate " ---> CCA "proline" , glutamate and proline are different amino acids , when the point mutation causes amino acid change in protein , that called "**mis sense mutation** " , this amino acid change may alter the functionality of protein , in fact there were three possibilities:
A. The protein will function "nearly" normally, in this case the new amino acid is Similar in chemical structure to the original amino acid , i.e if lycine replaced by arginine, this may not affect the functionality of the protein this type is Acceptable mis sense mutation . For example...
B. Protein will become less functioning, this type is partially acceptable mis sense mutation , for example.....
C. The function of protein lost , and this type is non acceptable miss sense mutation

3- in the gene "X" what happens when the codon CAA mutated and become TAA which is copyed in the mRNA as UAA and UAA is a stop codon . This will lead to premature termination of translation and premature protein will produced this type called **nonsense mutation**

Frame shift mutation

Frame shift mutation result from insertion or deletion of nucleotide or several nucleotides base pairs in a given location (figure 84) .the addition or deletion in DNA may or may not shift the reading frame of the mRNA i.e if we add a one or two nucleotide into Coding DNA region" and subsequently mRNA" the reading frame will shifted " remember that there is no punctuations between codons " (figure 84) , while the addition or deletion in non coding DNA will not cause shifting in the reading frame of mRNA , also addition or deletion of a three nucleotides "codon" will not cause shifting in the reading frame but will cause insertion or deletion of an amino acid on the protein .

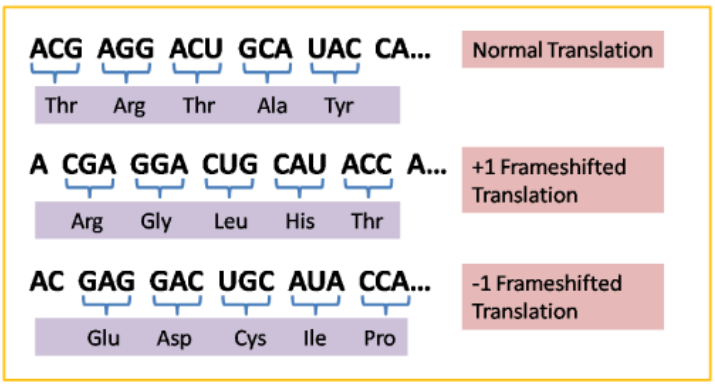

ACG AGG ACU GCA UAC CA...
Thr Arg Thr Ala Tyr
Normal Translation
A CGA GGA CUG CAU ACC A...
Arg Gly Leu His Thr
+1 Frameshifted Translation
AC GAG GAC UGC AUA CCA...
Glu Asp Cys Ile Pro
-1 Frameshifted Translation

figure 84

The addition or deletion may lead to creat a stop codon thus lead to premature termination of translation.

Theoretically if there is addition or deletion of thee nucleotides together , this will result in addition/deletion of an amino acid to/from protein , this case considered as **non-frame shift mutation** (Figure 85)

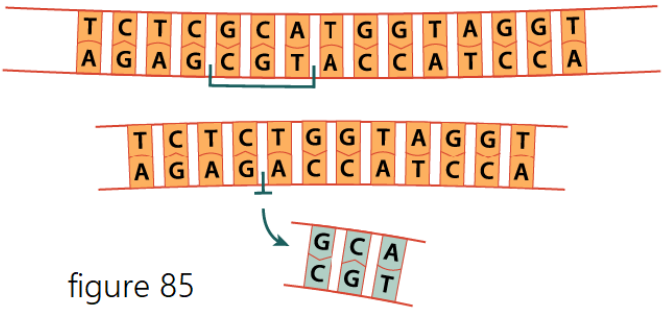


figure 85

DNA repair

The fundamental purpose of life is the transmission of genetic material from generation to generation without degradation or alteration .It is critical for living organisms to preserve genomic sequence information in order to continue their generations . DNA is frequently exposed to injurious agents " either endogenous or exogenous " that can cause a chemical or physical changes in DNA "DNA damage" like double and single chain breaks, insertions and deletions, Abasic sites, and DNA-protein cross-link formation as we will see

Difference between DNA damage and mutation

Both DNA damage and mutation are errors in The DNA , but the term damage is more comprehensive than mutation , However the damage in DNA can survive and pass to the next cell generation as mutation, the damaged DNA has a tendency for being mutated i.e if the damage is not detected and/or not repaired and the cell replicate this damaged DNA and divide ,the next generation of cells will be with a permanent Change in DNA .The frightening thing about DNA damage is not only the potentiality to be mutated ,the damage can also affect the process of gene expression . accumulation of DNA damage in muscles and neurons is one of the explanations of Aging process .

Chemical ,physical, biological agents can cause damage to the DNA . Repair is important to maintain the genetic material to survival of organism and species . Damaged DNA is often quickly detected and repaired by complex systems of repair

Causes of damage

Either Endogenous or exogenous sources can cause damage. Endogenous factors include spontaneous DNA mutations, reactive oxygen species (ROS) and lipid peroxidation products, endogenous alkylation agents, estrogen and cholesterol metabolites, and reactive carbonyl species . Exogenous factors include UV light, ionizing radiation, heavy metals, air pollution, cigarette smoke, and chemotherapy drugs. Every day, approximately 10,000 lesions accumulate in the DNA of each cell. This damaged DNA must be removed or repaired in order for the DNA code to be correctly read

Types of damage

I. Single base alteration

A.Missing base "AP site" , AP= apurinic/apyrimidinic (figure 87 a) are the result of cleavage of the bond linking the base to the deoxyribose in the sugar-phosphate backbone of DNA

B. Deamination of cytosine to uracil

C.deamination of adenine to hypoxanthine

D.alkylation of base "usually by methyl"

E. Base analog incorporation

II. Two-base alteration

UV light-induced Thymine-thymine dimer (figure 87 b)

III chain breaks

IV Cross-linkage (figure 87 c)
A. Intra-strand "between bases of the same strand
B. Inter-strands
C.DNA-protein cross

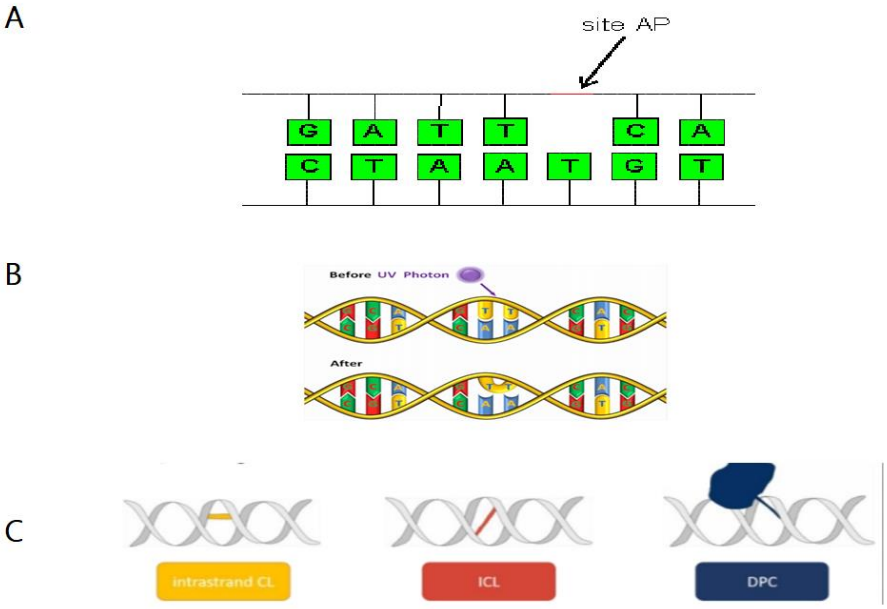


figure 87

Eukaryotic cells contain five major DNA repair pathways :
-Base excision repair "BER "

-Nucleotides excision repair "NER "

-Miss Matching repair" MMR"

-Homolgous recombination "HR"

-Non homologous end joining "NHEJ"

Note that the DNA repair mechanisms are sophisticated and It is not as easy as we will discuss This picture in (figure 88) is just to make you aware of its difficulty

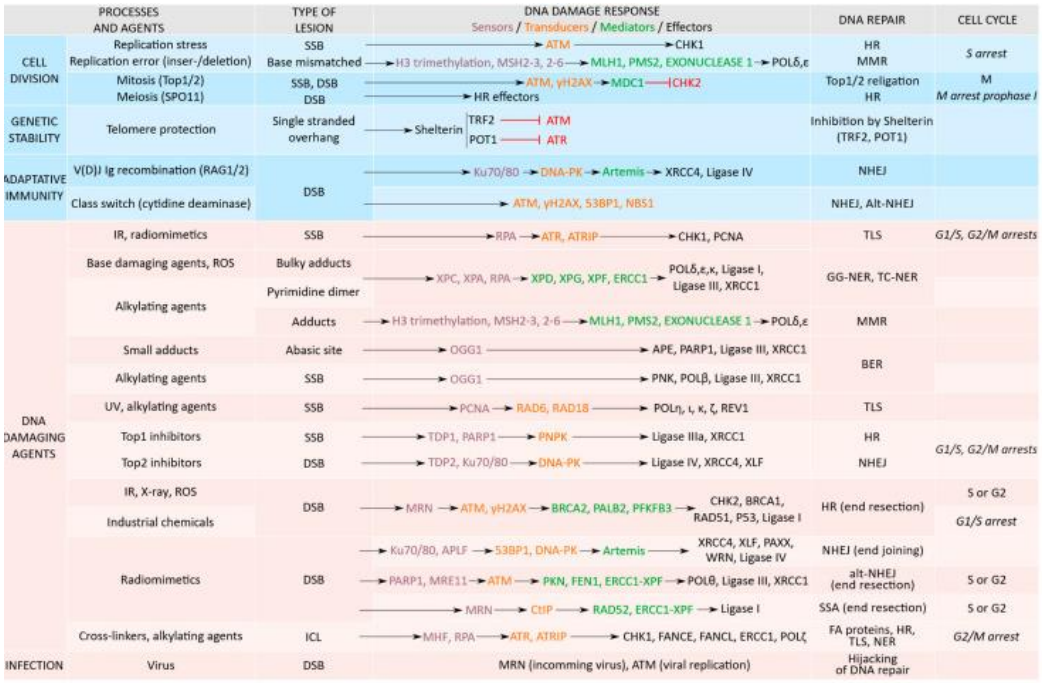


figure 88

BER

Base excision repair is a repair process that begins when a damaged base is removed through the action of **DNA N-glycosylase** that cuts the bond linking the base to the sugar-phosphate backbone of DNA (Figure89).This abasic (AP) site is then further processed by an AP endonuclease to cut the sugar-phosphate backbone. the small gap that remains is filled by a DNA polymerase and seals by ligase

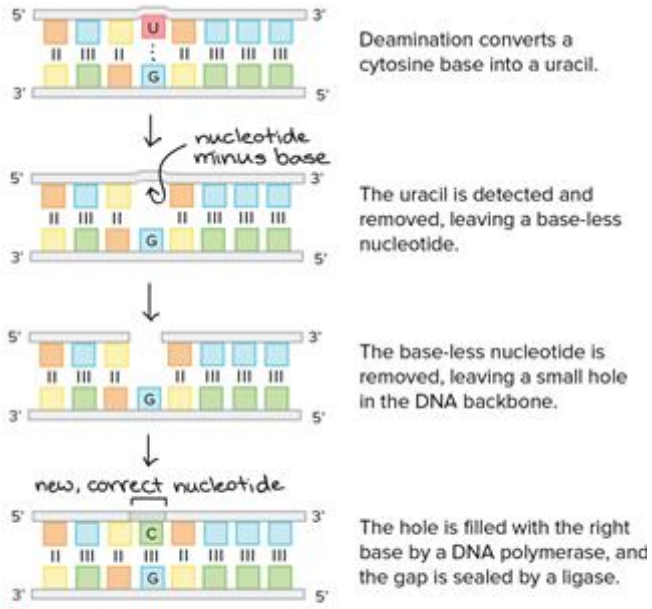

5'
3'
U
G
Deamination converts a cytosine base into a uracil.
nucleotide minus base
The uracil is detected and removed, leaving a base-less nucleotide.
The base-less nucleotide is removed, leaving a small hole in the DNA backbone.
new, correct nucleotide
C
The hole is filled with the right base by a DNA polymerase, and the gap is sealed by a ligase.

figure 89

NER

Responsible for repairing the two base alteration specially the T-T dimer (Figure90)

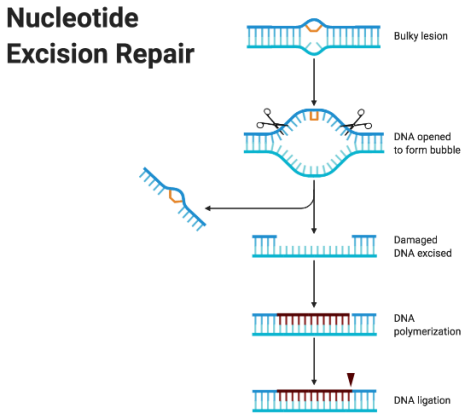


figure 90

Xeroeroderma pegmentoum

Xeroderma pigmentosum (XP) is a hereditary condition characterized by extreme sun sensitivity, leading to a very high risk of skin cancer and other medical problems. People with XP are extremely sensitive to ultra-violet (UV) radiation from the sun. The problem is in UV NER pathway

Mismatch repair :

The DNA damage that results during DNA replication is repaired by the MMR , in humans at least six different proteins are involved in MMR(Figure 91)

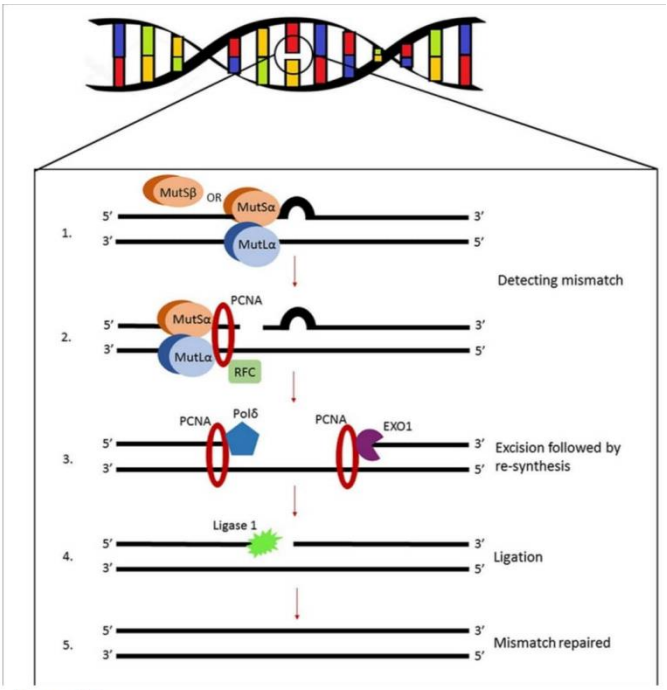


figure 91

HR and NHEJ

Both are used to repair DNA double strand breaks "DSBs", according to the phases of cell cycle either HR or NHEJ will selected, during G1/G0 phase of cell cycle the damage is usually corrected by NHEJ , while during M, S and G2 the damage is usually corrected by HR .

In HR pathway we use sister chromatid or homologous chromosome as template (figure 92)

In NHEJ we use many proteins to rejoining the DNA molecule " this after cuting the extensions that result from breaks" (figure 93) . In humans Ku (70,80) heterodimer , Artemis , XRCC4 and ligase VI are critical for NHEJ

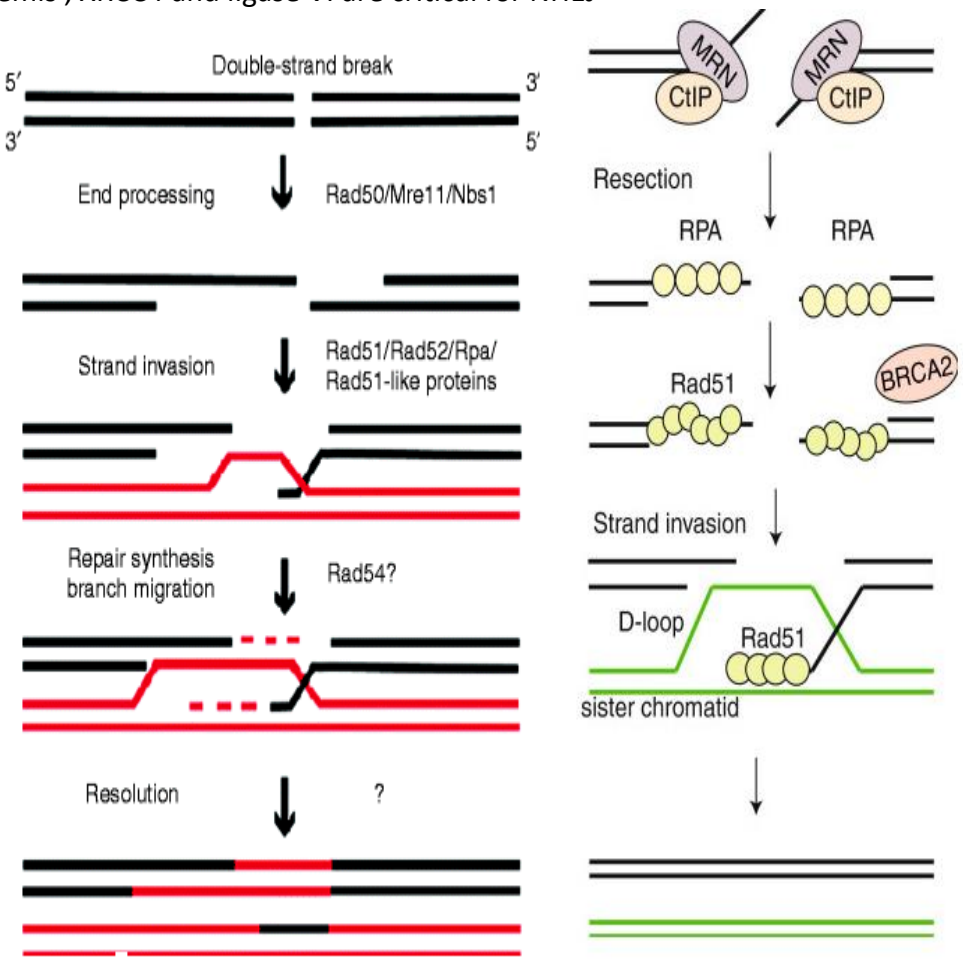


figure 91

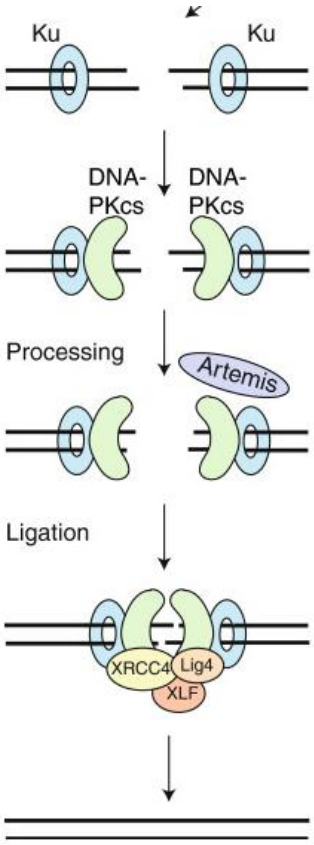

Ku
Ku
DNA-PKcs
DNA-PKcs
Processing
Artemis
Ligation
XRCC4
Lig4
XLF

figure 92

cell cycle and cancer

Cell cycle & cell cycle regulation

Cell cycle is a period of time that extend from the cell comes to existence (from pre-existing cell) to cell itself divide into two daughter cells .It gives a summarized picture for events that occur during cell life and often represent as pie chart (figure 94). In eukaryotic cells the cycle consist of two different phases :

- mitotic phase " period spent in division"
- interphase " period between divisions" also called intermitotic

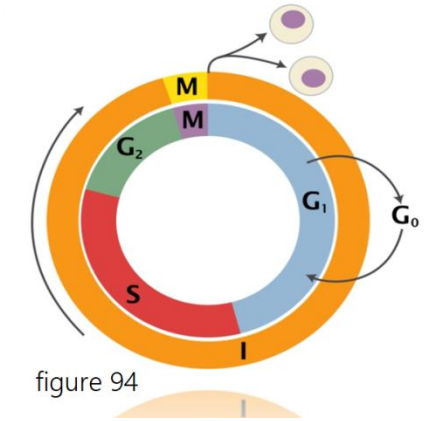


figure 94

Cells spend most of their time in the interphase when they growing ,accumulates nutrients that needed for division and replicate their DNAs and some of organelles .In other word the cell prepare to division in this phase

The inter phase consist of three phases , G1"first gap" , S"DNA synthesis phase" and G2 phase "second gap" . G1 and G2 are called gap phases because at first we thought that not much happens in gap periods , but actually incredible growth and synthetic activities "not DNA synthesis " take place .

In G1 phase the cell growing and prepares to DNA replication " by synthesis of enzymes and proteins needed for replication "DNA polymerase , histones ..etc "

In S phase DNA replication take place . In S phase there is very low rate of gene expression

In G2 phase cell undergoes more growth and prepare to mitosis " by synthesizing the enzymes and proteins needed for mitosis "microtubules , enzymes that degrade nuclear membrane… etc ".

In M phase the replicated chromosomes ,organelles and cytoplasm separate into 2 daughter cells

What about G0 ?! When the cell lack of the requirements of division or the cell itself non-dividable cell or haven't commitment to divide now or it only divide under specific conditions " semi non-dividable" enter a resting phase called "G zero" , And the cells perform their normal functions

Regulation of cell cycle

The Nobel Prize in Medicine or Physiology was recently "2001" awarded to three men, Leland Hartwell, Tim Hunt, and Paul Nurse, “for their discoveries of key regulators of the cell cycle”.

Cell cycle regulation

The proper transition from each cell cycle phase to the next is dependent on two classes of proteins called cyclins and cyclin-dependent kinases"CDKs" .

Actually the presence of proper amount of cyclins is determine cell progression because CDKs are constitutively expressed “ housekeeping genes” thus have a constant concentrations , in contract cyclins are synthesized at specific stages in response to specific molecular signals

Cyclins form complexes with CDKs and activate them to phosphorylates many substrates “either cause activation or inactivation” ultimately lead to progression in cell cycle .the stages of the cell cycle tend to have different cyclin-CDK complex (figure 95)

Cyclin	Kinase	Function
D	CDK4, CDK6	Progression past restriction point at G_1/S boundary
E, A	CDK2	Initiation of DNA synthesis in early S phase
B	CDK1	Transition from G_2 to M

figure 95

Example for how cyclins act

When the cell triggered to divide by mitogenic stimuli “GFs” the level of cyclin D increase , cyclin D then binds to CDK4/6 and activate it .Active cyclin-CDK complex phosphorylate many substrates “ either activate or in activate them”, one of this substrates is Rb "retinoplastoma" protein , Rb is transcription repressor found normally bound with transcription factor family E2F , phosphorylation of Rb cause E2F dissociation .E2F target the transcription of certain genes (histone genes, DNA replication proteins), and thus the cell can progress to DNA synthesis stage (figure 96)

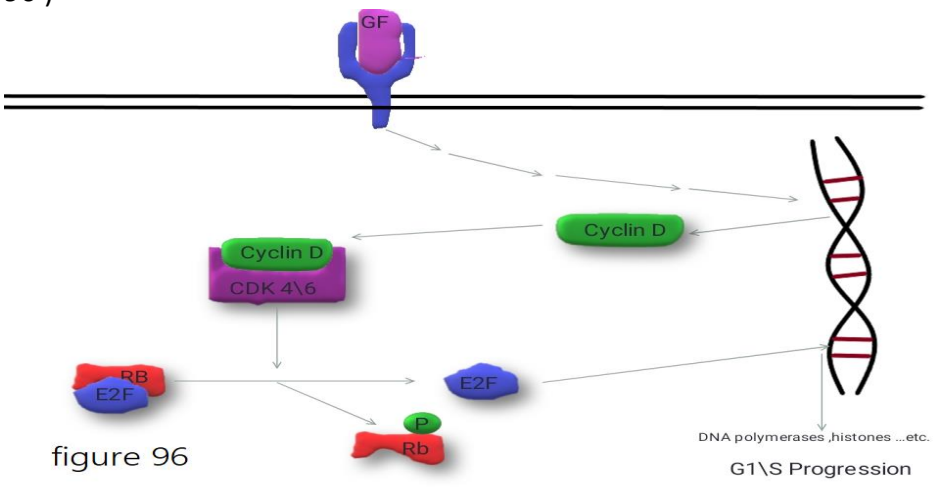


figure 96

Check points

A cell cycle checkpoint is a general term used to describe a cellular process that stops or slows the cell cycle . in conditions unfavorable for cell division, check points can be defended as network of proteins that monitor and regulate the cell cycle progression at different stages .

Simply we can say that ,the cell checks is it proper to goes to next stage ??? And there are three main checkpoints (figure 97)

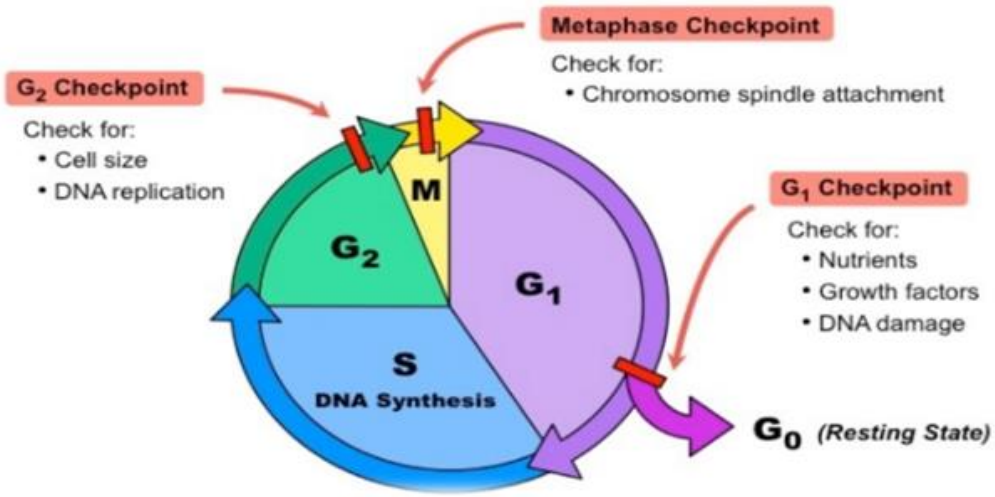


figure 97

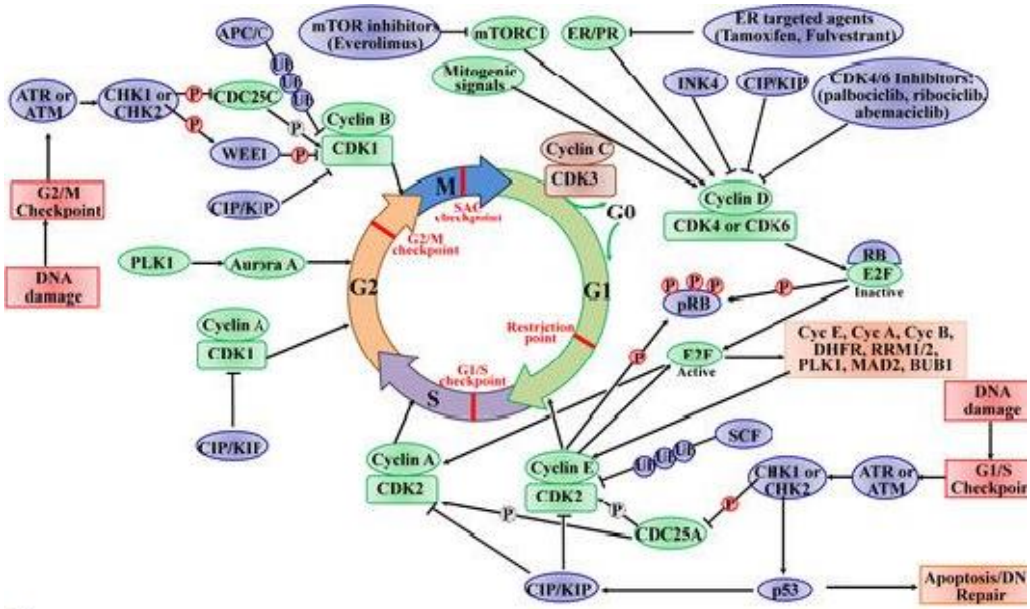


figure 98

figure 98 : complexity of cell cycle regulation

The

Cancer overview

The classic definition of cancer is “uncontrolled cell division.” In a large, multi-cellular organism, uncontrolled cell division will soon result in large masses of rapidly growing cells (tumors), which cause significant damage to surrounding tissues. When tumors spread, they can damage vital organs and eventually cause death.
It called cancer because the appearance of tumor with blood vessels surrounded it ,looks like a carb
the clinical features of cancer are determined by the location of cancer ,for example the caner of head of pancreas Vs breast cancer show different "not completely different" clinical features
Cancer is of clonally origin, with a single abnormal cell multiplying to become a mass of cells forming a tumor .Development of macroscopic tumor often take several years

Characteristics of cancer cell

Ability to rapid proliferation , loss of contact inhibition "in vitro" and developing anti-growing signals "in vivo" , Escape immunosurveillance by secrets large amount of mucin , Metastasis "most serious aspect" which is spreading by blood or lymphatic vessels and form a secondary tumors in other places, tumor cells and their surrounding cells able to secrete angiogenic growth factors because the low oxygen tension and thus there are stimulate new blood vessels formation " Stimulate local angiogenesis" , and cancer cell has Self-sufficient in growth signals

Causes

In generally cancer is a genetic disorder either caused by mutations in specific genes , or alterations in gene expression rates “ non mutational changes” of this specific genes .
Mutations can be inherited or acquired “ by exposed to many carcinogens such as chemical agents and radiations” .
Alterations in gene expression can also caused by carcinogens such as some onco-viruses , and some chemicals can cause alterations in epigenetic machinery.

It is impossible to summarize the causes of cancer , a lot of researches in this area , and oncology is a huge field . However we can say that, the cancer is a genetic disorder can be inherited or can be acquired by exposure to various of carcinogens

In general there are three classes of carcinogens :
-Radiant energy .(Ultraviolet, x-rays, ʎ rays)
-Carcinogenic chemicals.
-Oncogenic viruses

Radiant energy

Radiant energy , Such as UV rays , X rays and Gamma rays , the basic mechanism of carcinogenicity caused by radiation still under investigation .However as we see in the DNA damage and repair ,this agents can cause a damage in DNA in many ways "like T-T dimers , strand breaks …." and this damage can pass as mutations.
UV rays also induce the formation of Reactive Oxygen Species

Carcinogenic chemicals

A wide variety of chemical compounds are carcinogenic .Some chemicals interact directly with DNA , but others, termed procarcinogens, require conversion by enzyme action to become ultimate carcinogens (figure 99)

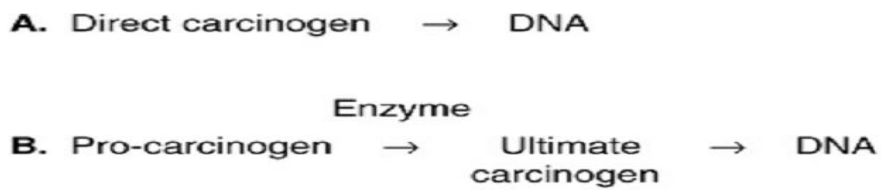


figure 99

Oncogenic viruses

Both DNA and RNA viruses have been identified as being able to cause cancer in humans .In general the genetic material of the virus incorporated into the genome of host cell ,that will results in various events that ultimately lead to cancer formation .

DNA viruses often act by down regulating expression of some tumor suppressor genes such as Rb or interferes with gene products of this tumor suppressor genes. RNA viruses often carry oncogenes in their genomes .

What are those "specific genes" that we were talking about early ?

There is two main classes of genes involved in carcinogenesis :

-**Oncogenes** "gas pedal" and **Tumor suppressor genes** "brakes" .
-tumor suppressor genes and oncogene
(Figure 100)

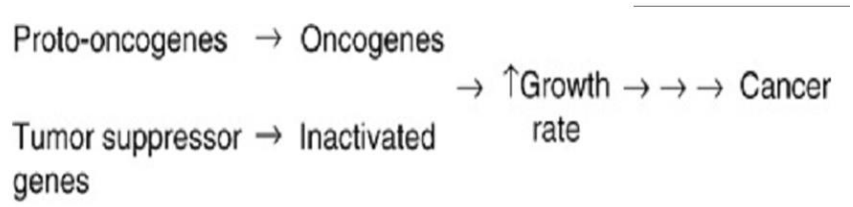


figure 100

Oncogenes

Oncogenes are Derived from proto-oncogenes ,which are normal genes encoded for products that trigger cell to divide “products ....not proteins only because some miRNAs seen as proto-oncogenes” .

If proto-oncogenes exposed to mutations that can make them “hyperactive” or there were over-expression in this genes ,they will transform to oncogenes ,and the cell will divide with abnormal rates . Genes that encoded for growth factors , their receptors and their signal transduction molecule, and positive cell cycle regulators such as cyclin D all are considered as proto-oncogenes because any hyperactivity in those genes eventually increase the rate of division.

Epidermal growth factor pathway as a model for understanding proto-oncogenes :

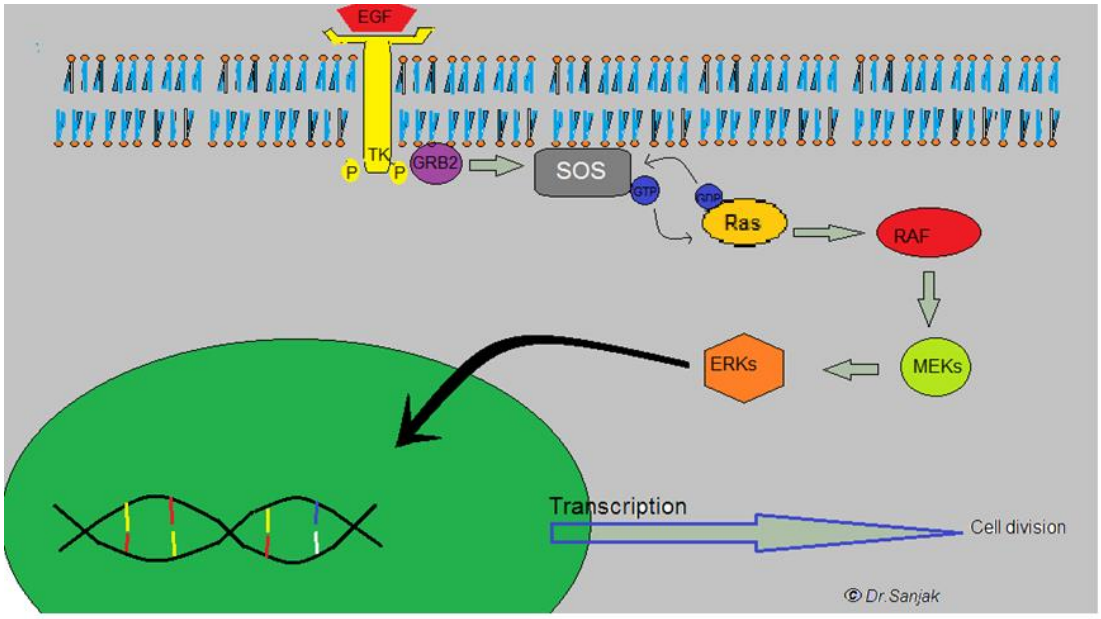


figure 101

1-Over-expression of EGF receptors and it ligands found in several human carcinomas

2- Loss of intrinsic GTPase activity of Ras makes it unable to swich off , thus lead to abnormal cell division. Ras is a family of genes encoded for small G-protein kinase active with GTP inactive with GDP , phosphorylate downstream substrates , it has intrinsic GTPase activity to terminate it actions ,and If this GTPase activity lost by a mutation the Ras protein become hyperactive

3-About 20% of oncogenes are oncogenic TFs

The figure 102 shows the ways of activation of proto-oncogenes

Mechanism	Explanation
Mutation	A classic example is point mutation of the *RAS* oncogene. This results in the gene product, a small GTPase, having less activity in tumors and in resultant stimulation of the activity of adenylyl cyclase.
Promoter insertion	Insertion of a viral promoter region near a gene activates it.
Enhancer insertion	Insertion of a viral enhancer region near a gene activates it.
Chromosomal translocation	The basis is that a piece of one chromosome is split off and joined to another. Classic examples are these involved in Burkitt lymphoma (see Figure 56–8) and in the Philadelphia chromosome (see the Glossary).
Gene amplification[a]	Abnormal multiplication of a gene occurs, resulting in many copies. This can occur with oncogenes and also genes involved in tumor drug resistance.

102

Note that the oncogenes act in a dominant manner
i.e if one mutated allele of Ras is presence"one defected Ras protein presence" that will enough to cause abnormal cellular division .

Tumor suppressor genes:

Here this genes produce products " also some miRNAs" that normally suppress the cell cycle , if the inhibitory effect is lost or diminished ..... the cell would be like a car without breaks .The most studied tumor suppressor genes are Rb and P53

P53 "guardian of the genome

P53 is a Gene encoded for protein with Mw= 53 kDA . P53 protein is a Transcription factor activates many genes related to DNA repair , cell cycle delay and also apoptosis .
P53 is one of the most frequently mutated genes in human cancers.

We can say , it the cancer's natural enemy

(Figure 103) Some differences between oncogenes & Tumor suppressor genes:-

Oncogenes	Tumor Suppressor Genes
Mutation in one of the two alleles is sufficient	Both alleles must be affected
Gain of function of a protein that signals cell division	Loss of function of a protein
Mutation arises in somatic cells, not inherited	Mutation present in germ cell (can be inherited), or in somatic cell
Some tissue preference	Often strong tissue preference (eg, effect of *RB* gene in the retina)

figure 103

Figure 104 Proprites of some proto-oncogenes and tumor suppressor genes

Name	Properties
MYC	An oncogene (OMIM 190080) that encodes a DNA-binding transcription factor, p53, that can alter transcription. Involved in cell growth, cell cycle progression, and DNA replication. Mutated in a variety of tumors.
P53	A tumor suppressor gene (OMIM 191170) that responds to various cellular stresses. It induces cell cycle arrest, apoptosis, senescence, DNA repair and is involved in some aspects of regulation of cellular metabolism. It has been named "the guardian of the genome." Mutated in some 50% of human tumors. The nomenclature p53 refers to the approximate molecular mass of the protein encoded by *P53*, as calculated from SDS-PAGE.
RAS	A family of oncogenes encoding small GTPases. They were initially identified as being the transforming genes of certain murine sarcoma viruses. Important members of the family are K-RAS (Kirsten), H-RAS (Harvey) (OMIM 190020), and N-RAS (neuroblastoma). Persistent activation of these genes due to mutations contributes to the development of a variety of cancers.
RB	A tumor suppressor gene (OMIM 180200) encoding the RB protein. RB regulates the cell cycle by binding the elongation factor E2F. It represses the transcription of various genes involved in the S phase of the cycle. Mutation of the *RB* gene is the cause of retinoblastoma, but it is also involved in the genesis of certain other tumors (see Chapter 35).

figure 104

The failure either in **DNA repair** or **apoptosis** may involved in developing and/or development of cancer cell , because failure in this mechanisms allow damages "and subsequently mutations" to accumulate ,and this is a supportive environment for cancer cell .

Epigenetic changes are increasingly being recognized in cancer .

Cancer cells and telomeres enzyme

Telomerase is often activated in cancer cells to enable cancer cells to duplicate their genomes indefinitely without losing important protein-coding DNA sequence.
Activation of telomerase could be part of the process that allows cancer cells to become immortal. The immortalizing factor of cancer via telomere lengthening due to telomerase has been proven to occur in 90% of all carcinogenic tumors in vivo with the remaining 10% using an alternative telomere maintenance route called ALT or Alternative Lengthening of Telomeres.

Metastasis

Metastasis is the movement or spreading of cancer cells from one organ or tissue to another. Cancer cells usually spread through the blood or the lymph system. If a cancer spreads, it is said to have "metastasized

Finally remember that ....

Fundamental features of carcinogenesis:-

-Nonlethal genetic damage is the initiating event in carcinogenesis, four classes of -genes, when affected , can result in the development of a tumor :

Proto-oncogenes.
Tumor suppressor genes.
Genes involved in DNA repair
Genes involved in apoptosis.

- Cancer is of clonally origin, with a single abnormal cell multiplying to become a mass of cells forming a tumor.
- Carcinogenesis is a multistep process, with multiple genetic alterations occurring in cells, transforming normal cells into malignant ones.

Tumor often takes several years to develop.

A stuent to student Guide

Cancer is a huge field and we aren't **oncologists** . We are modest 2nd year medical students

Regulation of gene expression " gene regulation"

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product . These gene products are proteins " through mRNA", and non-coding RNAs such as "rRNA , tRNA, snRNA ..." , But when we say gene expression , we mean proteins.

By altering the gene expression, organism can adapt to and overcomes environmental challenges .

Have you ever asked yourself why liver cells can't synthesize insulin and pancreas can't synthesize plasma proteins despite they share the same identical genome?
Simply these difference due to the tissue-specific gene expression, but why this selective expression occur at the first place ?
And how the cells differentiate and become specialized to show these specific patterns of gene expression ? In fact this is a deep issue
In biology and embryology .In simple the idea is different "not absolutely different" in gene expression among the tissues.

In both eukaryotes and prokaryotes the process of gene expression is under regulation. Dys- regulation of gene expression can lead to diseases such as cancer.

Some important terms

+Ve regulators or activators : something "specific regulatory proteins" cause increasing in gene expression
-ve regulators or silencers : something "specific regulatory proteins" cause decreasing in gene expression
Much of this regulation occurs at the level of transcription intitiation . Remember that the inhibition of the "-ve regulators" is a "+ve regulation"

Constitutive genes : genes expressed at constant rates , not known to be subjectd to regulation
Inducible genes : an inducible gene ,is a gene that his expression increase in response to activator "induction" .thus they called inducible genes , but those with high basal rate of expression are down regulated in response to inhibitors "repression" are called repressible genes , however the inducible genes may used to describe both genes that induced or repressed . inducible genes have variable levels of gene expression according to the cell status

Cistron : the smallest unit of gene expression can be polypeptide, protein subunit, or protein
In eukaryotes a single mRNA is encoded for single cistron , while prokaryotic mRNA can be polycistronic (figure 105)

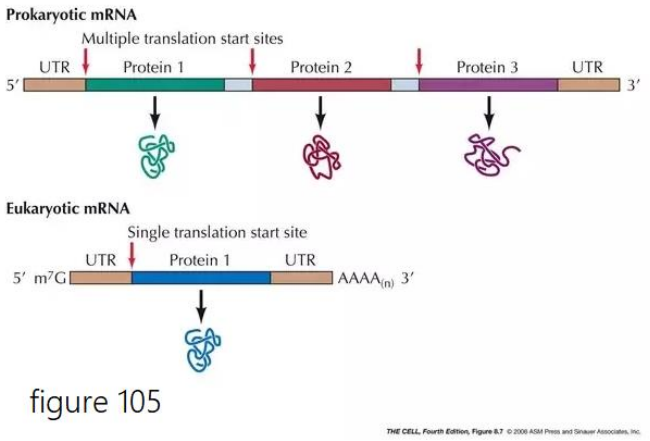


figure 105

Temporal responses

Biological systems exhibit three types of temporal responses to regulatory signals :

1.**Type A response**, there is increase in the extent of gene expression that dependent upon the continued present of inducing signal "such as hormone, nutrients, growth factors...." , Once inducing signal is removed, the amount of gene expression will return to the basal level .

2. **Type B response**: there is only a transient increase in gene expression even in the continued presence of signal , this occurs during the development of an organism when only transient appearance of gene product is required , also seen in the action of many pharmacologic agents

3. **Type C** : increase the extent of gene expression that persists indefinitely even after termination of the signal " irreversible inherited alterations" typically occurs during development of tissues and organs , this type of common in prokaryotes (Figure 106)

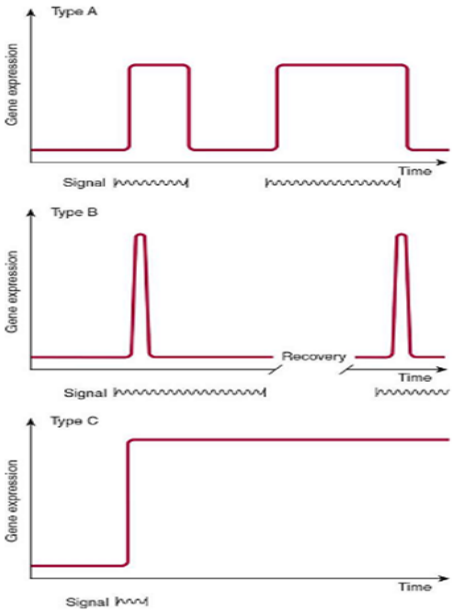


figure 106

Regulation of gene expression in prokaryotes:

Prokaryotic cells such as bacterial cells can regulate the gene expression by using a special types of proteins "DNA-binding proteins" . This proteins used by prokaryotes come in two categories , activators and repressors proteins which they can be controlled allosterically by other molecules .

Lac operon is a model for understanding gene regulation in prokaryotes

Analysis of lactose metabolism in E coli lead to discovery of the basic principles of gene transcription activators and repressors .
In prokaryotes, the genes that involved in metabolic pathways are often present in linear array called an operon e.g. Lac operon and tryptophan operon.
The operon consist of Coding site " for enzyme synthesis" and controlling sites "promoter and operator" (figure 107), operon also contain a regulatory gene "encoded for activator or repressor " that involved within operon .

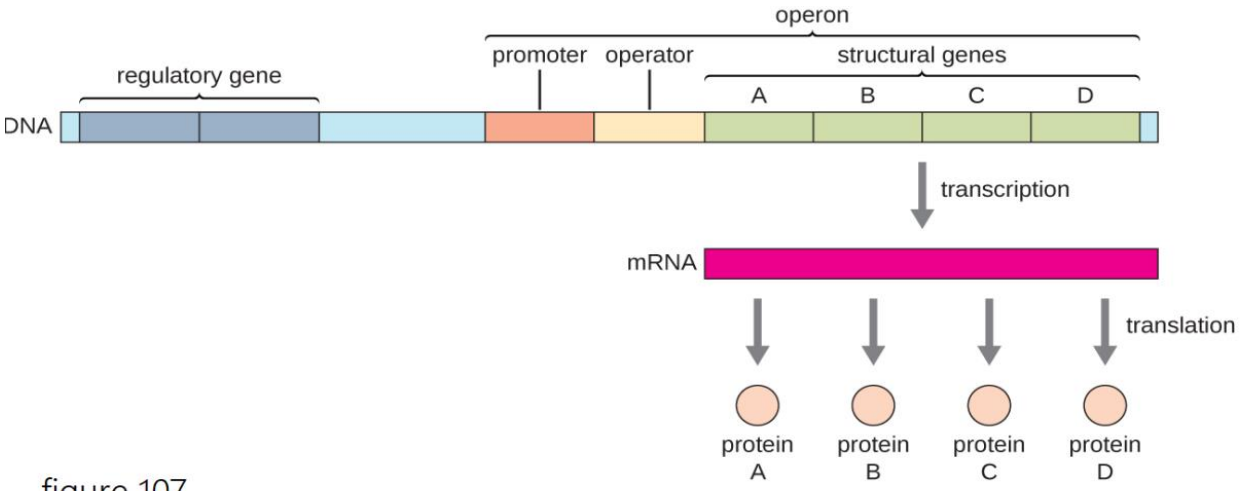


figure 107

Lac operon is an operon required for transport and metabolism of lactose , it has three adjacent structural genes Lac Z , Lac Y, and Lac A which are encoded for Bita-galactosidase , permiase and thiogalactosidase respectively . Lac Z, Y and A are transcribed into single large polycistronic mRNA molecule that contain multiple independent translation start sites and stop codons i.e. each protein translated separately and not processed from single large precursor (figure 108).

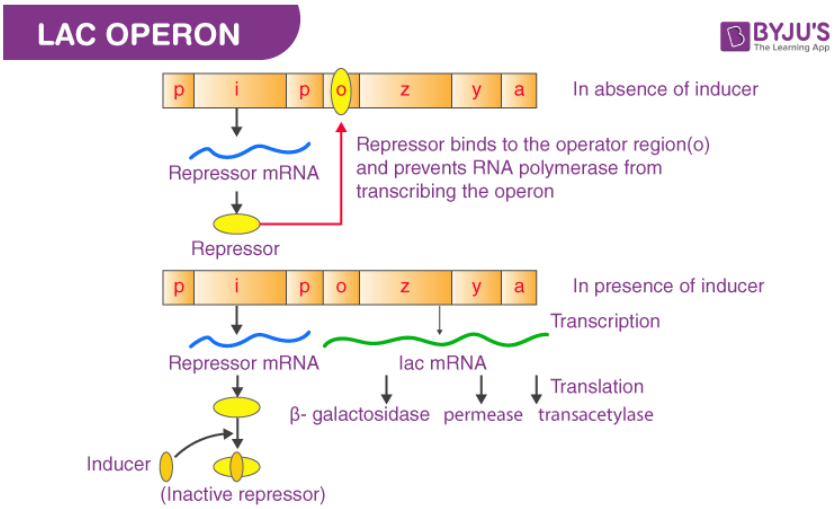


figure 108

By regulating lac operon , the E.coli and other enteric bacteria aim to prevent the synthesis of enzymes that related galactose metabolism since they not needed.
Normally this enzymes are synthesized only when two conditions are achieved :

1. **Presence of lactose**

2. With **absence of glucose** "or very low consentrition"
Once this condition achieved the synthesis of enzymes increase 100 - 1000 fold

Mechanism of lac operon regulation

In (figure 109 a) , Lac I is encoded for repessor protein binds with operator region and prevent the binding of RNA polymerase to the promoter , when no lactose is present

In (figure 109 b) , lactose or lactose analogs bind to repressor and inactivates it by decreasing repressor affinity to DNA "about 10,000 time" . Now RNA polymerase can bind to promoter region and initiate the transcripts but this relatively insufficient

In (figure 109 c) , the cell lack glucose and the cAMP start to accumulated within the bacterium "glucose inhibit Adenylyl cyclase" . cAMP bind to CAP "catabolic gene activator protein " and form cAMP-CAP complex , which bind to DNA just upstream of the promoter site, transcription occurs at maximal levels by unknown mechanism but may be by direct protein-protein interaction with RNA polymerase

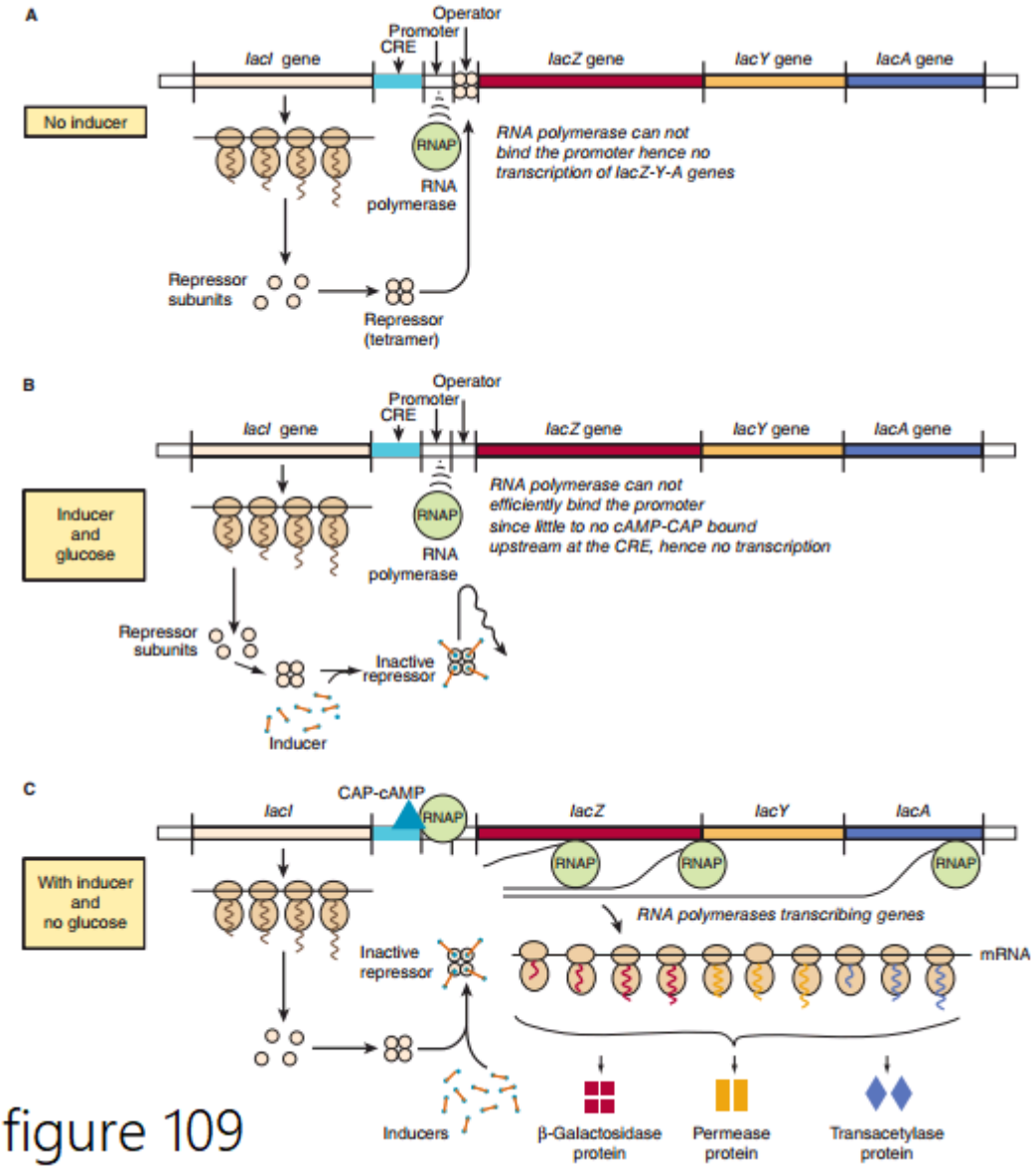


figure 109

Gene regulation in eukaryotes :

All organisms must regulate which genes are expressed at any given time.In multicellular organisms regulation of gene expression is essential for cell specialization.

Eukaryotic gene regulation is more complex because eukaryotes possess a nucleus.(transcription and translation are not coupled).

Eukaryotic gene expression is regulated at the following levels :

- DNA level " chromatin level"
- transcription level "most common"
-mRNA level "RNA processing, transport ,and degradation"
-translation level "also post translation level"
- protein degradation

(Figure 110)

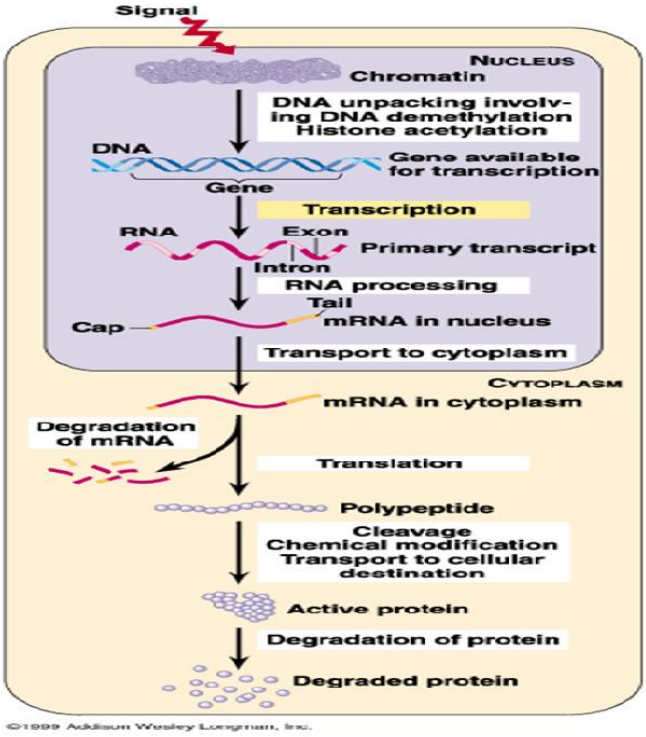


figure 110

We will focus on DNA and transcription levels .

DNA level "accessibility to DNA"

The DNA in eukaryotes is extensively folded and packed into protein-DNA complex called chromatin. Histones are important part of this complex, and contribute significantly in gene regulation.

The term **euchromatin** means transcriptionally active DNA which is less condensed and available for transcription.

Heterochromatin means that transcriptionally inactive DNA which is very condensed and not available for transcription , it can be :

- constitutive heterochromatin , which is always condensed and thus essentially inactive

- facultative heterochromatin , is at times condensed, but at other times it is actively transcribed and, thus, uncondensed and appears as euchromatin. Of the two members of the X chromosome pair in mammalian females, one X chromosome is almost completely inactive transcriptionally and is heterochromatic. However, the heterochromatic X chromosome decondenses during gametogenesis and becomes transcriptionally active during early embryogenesis—thus, it is facultative heterochromatin

Histone modifications

DNA of eukaryotic cells is tightly bound to small basic proteins, the histones, that package DNA in an orderly manner to form chromatin.

To facilitate the transcription of certain genes and silence the expression of others, the DNA and the core histone tails undergo covalent modifications, which are collectively studied under the heading of 'epigenetic mechanisms of gene regulation.

In **histone acetylation**, acetyl groups are added to positively charged lysine in histone tails , this decrease the positivity of histones ,and thus the interaction between DNA "negatively charged" and histones will decrease and loosens chromatin structure, thereby promotes the initiation of transcription and **increase** the gene expression.
There is also other modifications affect chromatin structure and subsequently gene expression, but histone acetylation and deacetylation are most understood.

DNA methylation

DNA methylation at specific deoxycytidine residues "CpG sites mainly at Promotors" is associated with reduction in transcription rate "methylation= gene silencing" in many species .

How does DNA methylation silence gene transcription?

-The nuclear matrix proteins, methyl-CpG-binding proteins 1 and 2, bind preferentially to the methylated cytosines. Presence of the methyl-CpG-binding proteins, bound to the methylated cytosines in the promoter region, obstructs binding of appropriate transcription factors

Actively transcribed genes are characterized by the presence of CpG sites in their promoter regions that are hypomethylated.
As the CpG sites in the promoter region are hypomethylated, it is possible for appropriate transcription factors to bind to their recognition sequences in the promoter .
in mouse liver, only the unmethylated ribosomal genes can be expressed, and there is evidence that many animal viruses are not transcribed when their DNA is methylated. Acute demethylation of 5MeC residues in specific regions of steroid hormone inducible genes has been associated with an increased rate of transcription of the gene. However, it is not yet possible to generalize that methylated DNA is transcriptionally inactive, that all inactive chromatin is methylated, or that active DNA is not methylated .

Gene amplification

Gene amplification means making copies of DNA "gene" , this will increase the product of that gene . Amplification of dihydro folate reductase gene in the resistance to cancer chemotherapy methotrexate is an example for gene amplification

Epigenetic mechanisms that involved in regulation of gene expression

Epigenetics is the study of heritable changes in chromatin without involving changes in the DNA sequence . The term epigenome "above the genome" describes epigenetic modifications all over the genome.

The epigenetic modifications alter the gene expression without altering in the underlying DNA sequence , such as DNA methylation and histone covalent modifications. Alterations in epigenetic factors may lead to diseases such as cancer . Epigenetic alterations also have been linked to addiction

Regulation at transcription level

The process of transcription is the first stage of gene expression resulting in the production of a primary RNA transcript from the DNA of a particular gene. It is the most common level for regulation.

Regulatory elements "DNA sequences" that regulate the transcription :

Promotors , which are Short nucleotides sequence serve as recognition point for RNA polymerase binding, there are located adjacent to the genes just few base pairs upstream , if promoter blocked by methyl groups the gene expression will reduced .

Enhancers , enhancer is a DNA sequence affect " increasing" the transcription of if specific gene , most enhancers are function in a tissues-specific manner , they serve as binding sites for the "activators" .(Figure 111 shows the difference between enhancer and promoters)

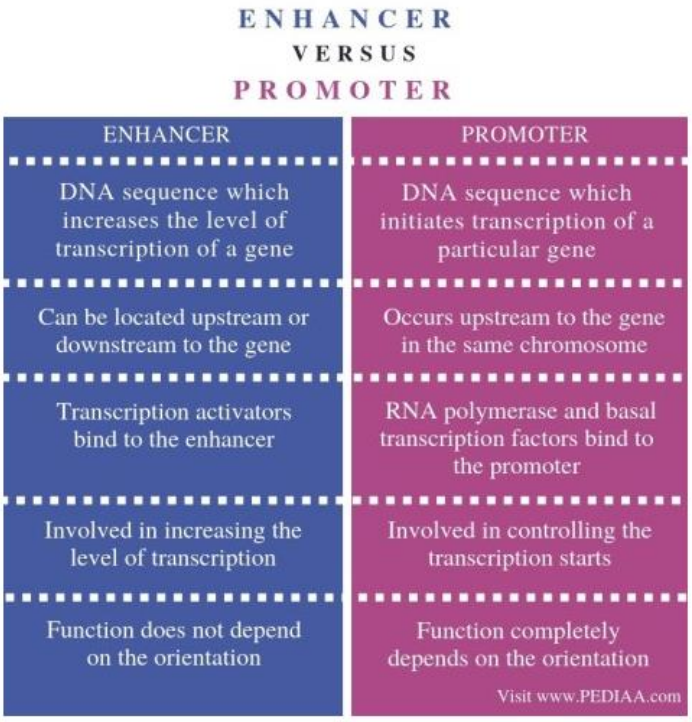

ENHANCER
VERSUS
PROMOTER
ENHANCER
PROMOTER
DNA sequence which increases the level of transcription of a gene
DNA sequence which initiates transcription of a particular gene
Can be located upstream or downstream to the gene
Occurs upstream to the gene in the same chromosome
Transcription activators bind to the enhancer
RNA polymerase and basal transcription factors bind to the promoter
Involved in increasing the level of transcription
Involved in controlling the transcription starts
Function does not depend on the orientation
Function completely depends on the orientation
Visit www.PEDIAA.com

figure 111

Mechanism of action of enhancers

Specific proteins bind to enhancer sequence and influence the activity of proteins that bind to promoter e.g. general transcription factors and RNA polymerase . Enhancer can be far from promoter several thousands nucleotides , how it regulate

the activity of a gene from this long distance? A bending factor bends the DNA and cause enhancer to be closer to promoter (figure 112)

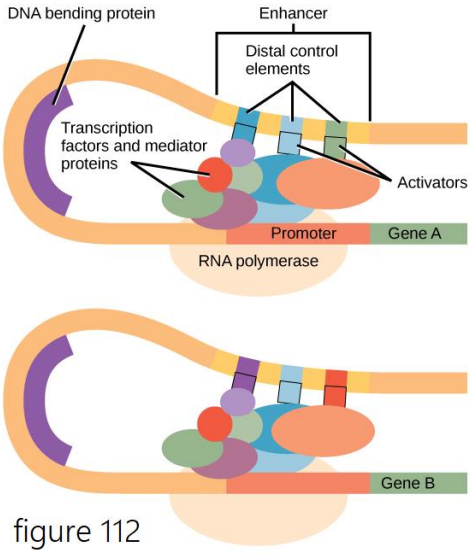


figure 112

Silencers , simply we can say that the silencers similar to enhancers , but they bind to repressors and cause transcription to be reduced .

The transcription factors

specific protein factors known as transcription factors bind to particular DNA sequences in gene regulatory regions and control their transcription. These transcription factors are commonly classified into families on the basis of the precise protein structure which they use to mediate binding to DNA . The function of transcription factors is to regulate - turn on/off - the transcription of genes .

Generally transcription factors are consist of two domains , DNA binding domain and transcriptional activating domain " for binding to co-regulatory proteins" , some transcription factors have third domain called signal sensing domain "for binding to signaling molecules"(figure 113)

the majority of transcription factors act in a positive manner. number of cases have now been described in which a transcription factor exerts an inhibitory effect on transcription , the interplay of positively and negatively acting transcription factors has a critical role in controlling the process of gene expression.

We have two types of transcription factors , general "basal" transcription factor (see transcription) , and special transcription factors which regulate "increase or decrease" only special genes and they bind to enhancer regions and they are tissue-specific

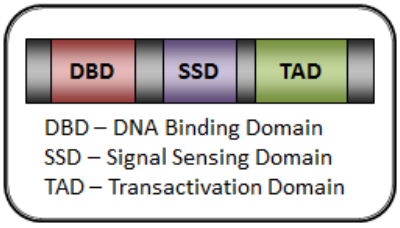


figure 113

DNA binding motifs

Are super secondary structures that help the binding of regulatory proteins to the right DNA regions such as Zinc finger motif , Lucien zipper motif and helix-turn-helix motif.

Environmental and biological factors that affect the transcription

Environmental factors can alter the rate by which transcription occur in a cell , for example when a plant cell exposed to sun light this ultimately leads to increase the gene transcription of ribolluse carboxylase which is important in photosynthesis process .

Biological factors such as hormones, are also alter the transcription of specific genes . Either group I "such as steroids" or group II "such as insulin" can alter the expression of specific genes . Steroids are easily pass through the cell membrane and then bind to intracellular receptors to form hormone-receptors complexes which can interact with the regulatory elements of the DNA and regulate the transcription .

Peptide hormones cannot pass through cell membrane easily, so convey signals through membrane bound receptors,the hormone binds receptor on cell surface, signal gets internalized, then cascade of events begin

Overview on PCR

Gel electrophoresis

Gel electrophoresis is a technique commonly used in laboratories to separate charged molecules like DNA, RNA and proteins according to their size.
Charged molecules move through a gel when an electric current is passed across it.
An electric current is applied across the gel "usually agarose gel" so that one end of the gel has a positive charge and the other end has a negative charge.
The movement of charged molecules is called migration. Molecules migrate towards the opposite charge. A molecule with a negative charge will therefore be pulled towards the positive end (opposites attract!) (Figure 117).
Electrophoresis enables you to distinguish DNA fragments of different lengths. and it has many applications in forensic medicine and diagnosis of genetic diseases.

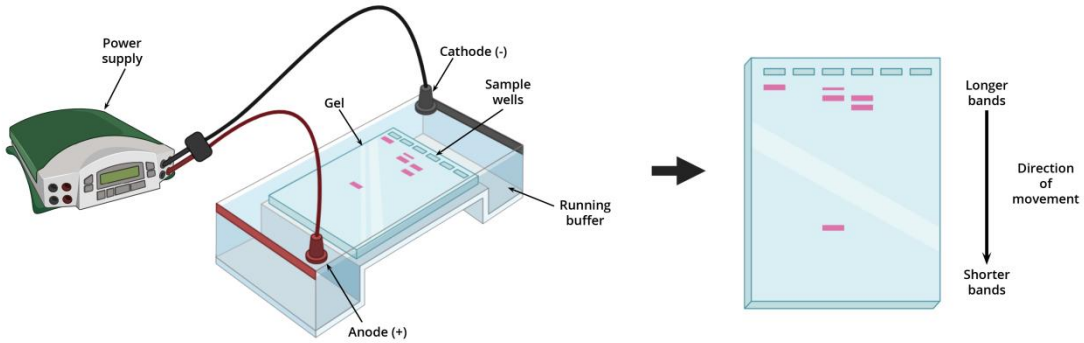


figure 117

PCR

An extremely powerful technique that is based on the concepts we have been considering in this chapter is
the Polymerase Chain Reaction or PCR. PCR makes it possible to amplify a desired of segments of DNA "
. The DNA is amplified in an exponential fashion, meaning that each cycle of
PCR, in principle, doubles the number of DNA molecules with a specific sequence present in a test tube. As
a result, minute quantities of DNA, as little as a single molecule, can be amplified into billions of molecules
in just a few hours using relatively simple equipment. PCR was invented by Kary Mullis, who earned the
1993 Nobel Prize in Chemistry for his achievement

requirements of PCR

-A piece of DNA template (needed to be amplified).

The four nucleotide bases dATP,

-dTTP, dGTP and dCTP (large quantities).

-A primer sequence (large quantities), which is small pieces of

-DNA, 15-30 nucleotides. It binds to

-DNA at specific region.

-Heat-stable DNA polymerase

-Thermocycler machine

Steps

1. The double stranded DNA is heated at 95°c for about 15 seconds , this breaks the hydrogen bonds that hold the strands together, thereby separating them .
2. Hybridization of primes "primer annealing"

The solution is cool down to about 54c° and DNA primers are added .the least of temperature allows hybridization to occur , one primer binds to the 3`end of one strand, and the other primer binds to the 3` end of the complimentary strand . Remember that in the DNA replication the DNA polymerase require a RNA primer to initiate the synthesis of new strand, in PCR also primer is required but we use DNA primer .

3. DNA synthesis " elongation"

dNTPs and specific temperature-resistant DNA polymerase called Taq " Termous aquaticus " DNA polymerase are added . The temperature increased to optimal 72°c . The DNA polymerase elongates the primers in 5`to3` direction

(Figure 118) illustrates the PCR process for the amplification of a segment of DNA

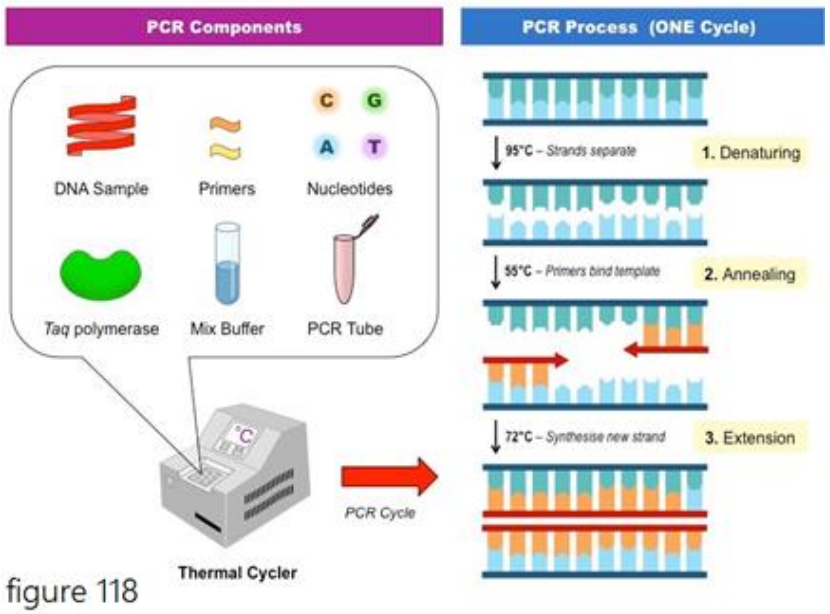


figure 118

After one cycle of PCR there are two copies of DNA "desired DNA" .... After two cycles will be four copies..... After three cycles will be eight copies. ..and so forth

2^n $(n = number\ of\ cycles)$ = total copies of desired DNA
We can do 25-40 cycle per hour

Applications of PCR

-Easy way to amplifying DNA in short time
- Detection of infectious agents e.g. HIV (AIDS) virus, and hepatitis (B and C) viruses, and bacterial bacilli of tuberculosis.
-Diagnosis of prenatal 'genetic diseases. Sample is obtained by amniocentesis (amniotic fluid by trans-abdominal needle).
- Forensic medicine: PCR allows the DNA in a single cell, hair follicle or sperm to be amplified and analyzed. Thus the application of PCR in crimes and rape is clear.

Recombinant DNA

Recombinant DNA Technology is another way for DNA amplification, in contract to PCR "DNA replication in test tubes" the recombinant DNA Technology involves replication of DNA within a living cell .

When we say recombinant DNA we mean ,a DNA price that has been created by combination of two "or more" DNAs from different sources, recombinant DNA is also called chimeric DNA . The chimeric DNA is formed artificially by combining constituents form different organisms , For example DNA segment"gene" from human inserted and combining with bacterial DNA .The idea of recombinant DNA was first proposed by Peter Lobban .

Applications

-Production of proteins for treatment, research and diagnosis.
-Production of genes of interest for researches and other purposes e.g. insulin gene.
- Gene mapping.
- Gene therapy.
- Molecular analysis of diseases (pedigree analysis).
-Diagnosis of fetal genetic disorders
The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences .Recombinant DNA is used to identify, map and sequence genes

DNA recombinant is possible because DNA molecules from all organisms share the same chemical structure, differing only in the nucleotide sequence

Requirements

-The DNA of interest "desired DNA"
-Victor "host DNA"
-Restriction enzymes
-DNA ligase

Restriction enzymes

One way that bacterial cells protect themselves from bacteriophages "bacterial viruses " is by using special proteins called restriction enzymes " exonucleases" can cut the viral DNA at specific locations , thus there are defensive mechanism protect the bacterial cell from DNA of the foreign organism" primary infective phages" .
Restriction enzymes found only in cells only in cells that have an accompanying enzyme which methylates the host DNA, rendering it unsuitable substrate for digestion by restriction enzymes.

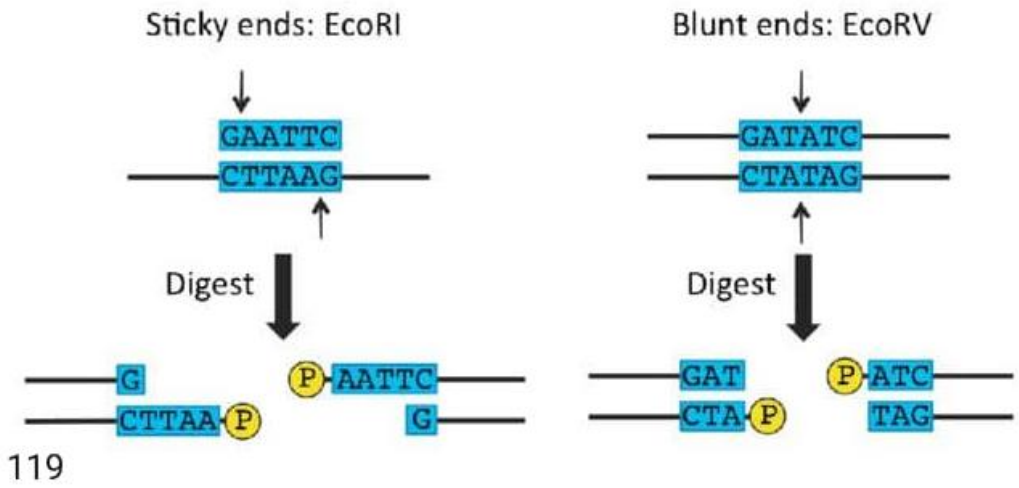
The restriction enzymes are a key tool in recombinant DNA recearchs , there are used to prepare chimeric DNA molecule .they cut DNA of any source into unique

short prices in a sequence-specific manner in contract to most other enzymatic, chemical, or physical methods, which break DNA randomly .
Each enzyme recognizes & cleaves a specific double-stranded DNA sequence.
These DNA cuts result in either:

(1**) blunt** ends

(2)**sticky**, cohesive or staggered ends.

Sticky ends are particularly useful in constructing hybrid or chimeric DNA molecules (Figure119).



119

In the (figure120) when we use the same restriction enzyme on two different DNA molecules this allows the DNA of interest "human DNA segment" to incorporate within the host DNA "bacterial DNA" by helping of DNA ligase .

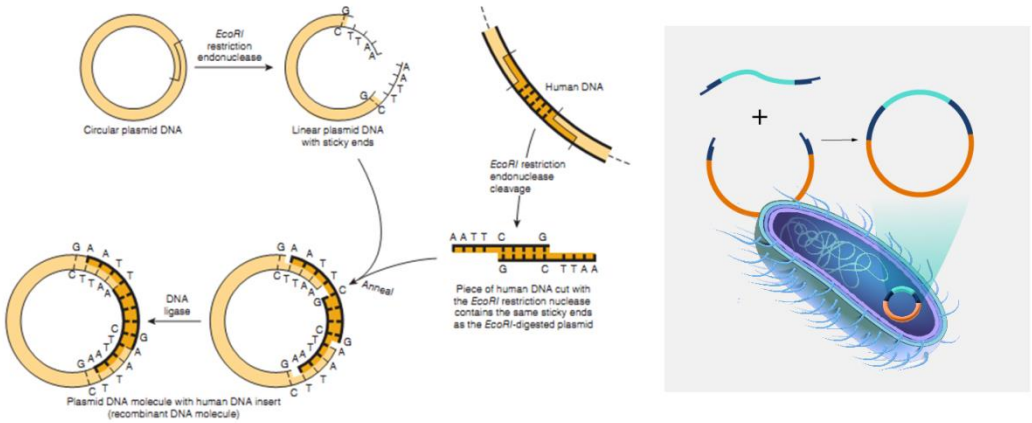

EcoRI restriction endonuclease
Circular plasmid DNA
Linear plasmid DNA with sticky ends
Human DNA
EcoRI restriction endonuclease cleavage
Piece of human DNA cut with the EcoRI restriction nuclease contains the same sticky ends as the EcoRI-digested plasmid
Anneal
DNA ligase
Plasmid DNA molecule with human DNA insert (recombinant DNA molecule)

figure 120

clone is a large population of identical molecules, bacteria, or cells that arise from a common ancestor. Molecular cloning allows for the production of a large number of identical DNA molecules, which can then be characterized or used for other purposes. This technique is based on the fact that chimeric or hybrid DNA molecules can be constructed in cloning vectors—typically bacterial plasmids, phages, or -cosmids—which then continue to replicate in a host cell under their own control systems. In this way, the chimeric DNA is amplified.

Colin vectors

Plasmids

Small, circular, duplex DNA molecules, natural function is to confer antibiotic resistance to the host cell. Exist as single or multiple copies within the bacterium .Replicate independently from the bacterial DNA. The complete DNA sequence of many plasmids is known.

Phages " lambda phage"

Lambda phage is a special type of bacteriophage infects the E.coli bacteria , it has linear DNA molecules into which foreign DNA can be inserted at several restriction enzyme sites.

A major advantage of phage vectors is that its accept DNA pieces larger than plasmid.

While plasmids can accept DNA pieces [6–10 kb long], phages[10–20 kb long]

Cosmids

A cosmid is a hybrid vector containing the usable features of plasmids and phages , it can accept DNA parts for cloning until 37,000 to 52,000 base pairs.

There is other cloning vectors such as bacterial artificial chromosome "BAC" , yeast artificial chromosome " YAC " and E. coli bacteriophage P1-based (PAC) vectors.

These vectors will accept & propagate DNA inserts of several hundred kilobases or more & replaced plasmid, phage &cosmid vectors for some cloning & gene mapping applications.

The expression vectors

A vector in which the protein coded by the gene introduced by recombinant DNA technology is actually synthesized .

Note

When desired DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences

Insulin production as a model to understand the usefulness of recombinant DNA Technology :

-The DNA for insulin is first isolated " in fact we use cDNA that formed from mRNA of insulin "

-A plasmid made of DNA is removed from a bacterial cell

-A restriction enzyme cuts the plasmid DNA open, leaving sticky ends.

-The insulin gene, with complementary sticky ends is added.

-DNA ligase enzyme splices (joins) together the plasmid DNA and the insulin DNA.

-The plasmid (now genetically modified) is inserted back into the bacterium.

-The bacterium host cell, divides and produces copies of the plasmid.

- The Bacterium makes human insulin using the gene in the plasmid.

-The insulin is extracted from the bacterial culture.

(Figure 121)

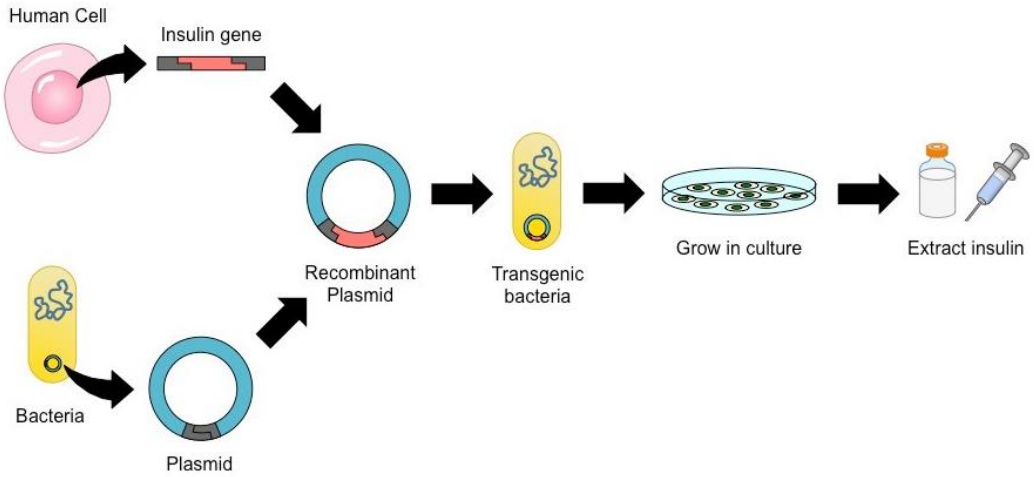


figure 121

Chapter 2 :

Xenobiotics metabolism overview

Xenobiotics

xenobiotics are understood as substances foreign to an entire biological system . The term xenobiotic is derived from the Greek words ξένος (xenos) = stranger.

The principal classes of xenobiotics of medical relevance are drugs, chemical carcinogens, naturally occurring compounds in plant foods, and various compounds that have found their way into our environment by one route or another. Here in this chapter will focus on Drugs metabolism.

Drugs metabolism "biotransformation"

Drug metabolism is the biochemical modification of pharmaceutical substances or "xenobiotics" by living organisms, usually through specialized enzymatic systems.
.The Aim is to make drugs more hydrophilic to be easily excreted , these metabolism can result in four possibilities :
-Active compound converted to inactive one
-Active compound converted to still active one
-Inactive compound "pro drugs and pro carcinogens" converted to active one
-Active/inactive one converted to a toxic compound ,

are all drugs undergo metabolism? In fact no , the class of drugs called "Hard drugs" are already soluble so they are not undergo biotransformation.

Metabolism of Drugs is generally divided into Two Phases. In **Phase I**, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics.
These modified compounds are then conjugated to polar compounds in **Phase II** reactions.
These reactions are catalyzed by transferase enzymes such as glutathione Stransferase.

Phase 1 metabolism renders compounds more reactive, introducing groups that can be conjugated with glucouronic acid, sulfate, acetate, glutathione, or amino acids in **phase 2** metabolism. This produces polar compounds that are water soluble and can therefore readily be excreted in urine or bile.

Very hydrophobic xenobiotics would persist in adipose tissue almost indefinitely if they were not converted to more polar forms.
The rate of metabolism determines the duration and intensity of a drug's pharmacological action .

Phase I

In Phase I, a variety of enzymes acts to introduce reactive and polar groups into their substrates. One of the most common modifications is hydroxylation which catalyzed by a family of enzymes known as mono-oxygenases or cytochromes P450In Phase I, a variety of enzymes acts to introduce reactive and polar groups into their substrates.

One of the most common modifications is hydroxylation catalyzed by a family of enzymes known as mono-oxygenases or cytochromes P450 .

Cytocrome P450

Cytochrome p450 is a Super family of hemoproteins "heme containing enzymes" i.e it is super family consist of families "family 1, family 2, 3.." , the family consist of sub families "A, B , C ..." a subfamily contains members " member 1,2,3.." for example ,When we say CYP3A4, we mean the member number four in the sub family A of in the family three , however the CYP3A4 is the most important enzyme involved in drugs metabolism .However
The families CYP1 , CYP2 and CYP 3 are the major cytochromes in drugs metabolism.
CYP 450 enzymes are present nearly in all tissues ,but mainly in the liver , and they founded mainly in form of membrane bounded vesicles in the smooth endoplasmic reticulum "microsomal fractions" .

Because CYP 450 enzymes are overlapping in specificity and Some drugs considered as microsomal inducers i.e. increase e the rate of synthesis of CYP that metabolize it "which may use to metabolize other drugs " and other drugs inhibit the activities of CYP 450 enzymes that metabolize them "which may use to metabolize of other drugs" , this creat a problem should be overcame when a patient take two or more drugs metabolized by the same CYP enzyme , for example anticoagulant warfarin is metabolized by CYP2C9, which is

induced by phenobarbital. Induction of CYP2C9 by phenobarbital will increase the metabolism of warfarin, so reducing its efficacy, and the dose must be increased. In contract the Ceproflxcin inhibit the CYP2C9 so increasing the efficacy of warfarin, and the dose must be decreased, however we will see this in details in pharmacology.

The overall reaction catalyzed by a cytochrome P450 is in (figure 2-1)

Overall reaction

$$R\text{-}H + O_2 + NADPH + H^+ \longrightarrow R\text{-}OH + H_2O + NADP^+$$

figure 2-1

The role of NADPH is to reduce cytochrome P450; the reduced cytochrome then reduces oxygen to water and the hydroxyl group that is introduced into the substrate

Phase II

In phase II the hydroxylated xenobiotics produced in phase I are conjugated with molecules such as glucuronic

acid, sulfate, or glutathione. This renders them even more water-soluble, and they are eventually excreted in the urine or bile in Phase II
In phase II the hydroxylated xenobiotics produced in phase I are conjugated with molecules such as glucuronic acid, sulfate, or glutathione. This renders them even more water-soluble, and they are eventually excreted in the urine or bile

There is also some drugs that increase/decrease the rate of conjugation but that is less common

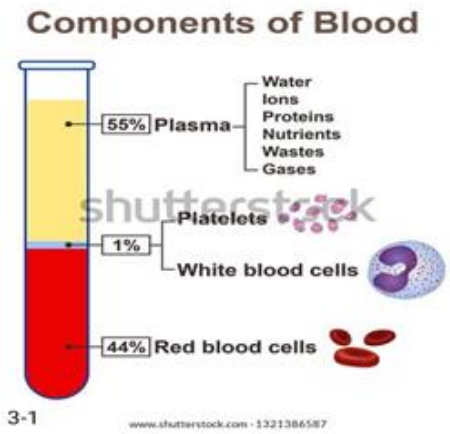
Chapter 3 :

Plasma proteins Chemistry, Functions and Clinical Significance

Introduction

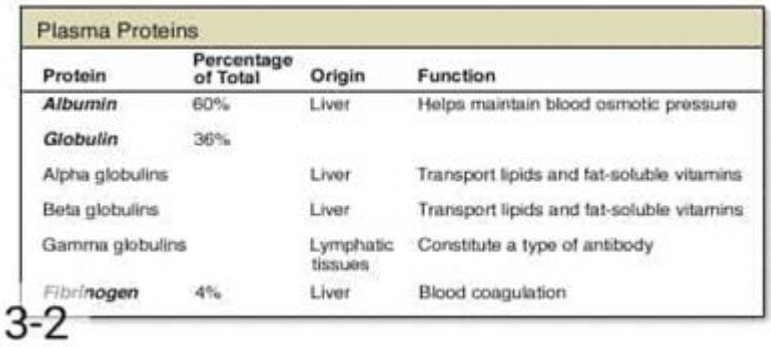
Plasma proteins are the collection of intricate molecules found in blood plasma. Plasma proteins serve many functions , and they are mostly synthesized by the liver. Albumin, globulins, and fibrinogen are the three most important plasma proteins
These proteins carry out a number of tasks, include regulation of the blood's osmotic pressure, transporting of hormones and other chemicals, and assisting in the formation of blood clots.
The definition, purposes, obligations, activities, advantages, and disadvantages of plasma proteins will all be covered in this article.

Plasma consists of water, electrolytes, metabolites, nutrients ،proteins, and hormones.(figure 3-1)
The concentration of total protein in human plasma is approximately 6-8 g/dL.
The proteins of the plasma are a complex mixture that includes
1- **Simple proteins**
2- **Conjugated proteins such as glycoproteins and various types of lipoproteins**.



The plasma proteins consist of :

-Albumin (3.5 to 5 g/dl)
-Globulins (2.5 to 3.5 g/dl)
-Fibrinogen (200-400mg/dl)
(Figure3-2)
The albumin: globulin (A/G) ratio is usually between 1.2 to 1.5



Plasma Proteins

Protein	Percentage of Total	Origin	Function
Albumin	60%	Liver	Helps maintain blood osmotic pressure
Globulin	36%		
Alpha globulins		Liver	Transport lipids and fat-soluble vitamins
Beta globulins		Liver	Transport lipids and fat-soluble vitamins
Gamma globulins		Lymphatic tissues	Constitute a type of antibody
Fibrinogen	4%	Liver	Blood coagulation

3-2

Plasma proteins are synthesized in the liver "Y-globulins - synthesized in plasma cells too " .
-They Synthesized as pre-proteins on membrane-bound polyribosomes: then they are subjected to posttranslational modifications in endoplasmic reticulum &Golgi apparatus
-Almost all of them are glycoproteins " albumin is an exception "
They have characteristic half-life (e.g albumin = 20 days)

Functions of plasma proteins

- Blood coagulation
-maintenance of homeostasis (pH, osmotic pressure)
-Defense against infection
-Transport of hormone, nutrients, drug ,metabolites and metabolic waste product

Separation of plasma proteins : -
By using the Salting out methods they classified into three major groups , fibrinogen, albumin and globulins . We use a vary concentrations of sodium or ammonium sulfate in this method.

By use **Electrophoresis** they classified into five major fractions :
-Albumin
-α globulins & α2 globulins
-ß globulins
-Y globulins

Albumin (69 kDa)

is the major protein of human plasma (3.5-5 g/dL)
Makes up approximately 60% of the total plasma proteins .the . half life of albumin is about 20 days . albumin Migrates fastest in electrophoresis at alkaline pH and precipitates last in salting out methods .

Synthesis of Albumin

The liver produces about 12 g of albumin per day, representing about 25% of total hepatic protein synthesis.
Albumin is initially synthesized as pre-pro-protein. Mature human albumin consists of one polypeptide chain of 585 amino acids and contains 17 disulfide bonds .

It has an ellipsoidal shape, which means that it does not increase the viscosity of the plasma as much as an elongated molecule such as fibrinogen does

Functions of Albumin

- Colloidal osmotic Pressure : albumin is responsible for 75-80% of the osmotic pressure of human plasma due to its low molecular weight and high concentration,
lt plays a predominant role in maintaining blood volume and body fluid distribution
Hypoalbuminemia leads to retention of fluid in the tissue spaces manifested by (Edema)
-Transporting function: Albumin has an ability to bind various ligands, thus acts as a transporter for various molecules. albumin is the carrier of :
-Copper
- free fatty acids (FFA),Bilirubin and certain steroid hormones .
-variety of drugs, including sulfonamides, penicillin G, phenytoin and are also bound to albumin

-Nutritive Function : Albumin serves as a source of amino acids for tissue protein synthesis to a limited extent, particularly in nutritional deprivation of amino acids.
Buffering Function: Among the plasma proteins, albumin has the highest buffering capacity due to its high concentration and the presence of large number of histidine residues, which contribute maximally towards maintenance of acid base balance.

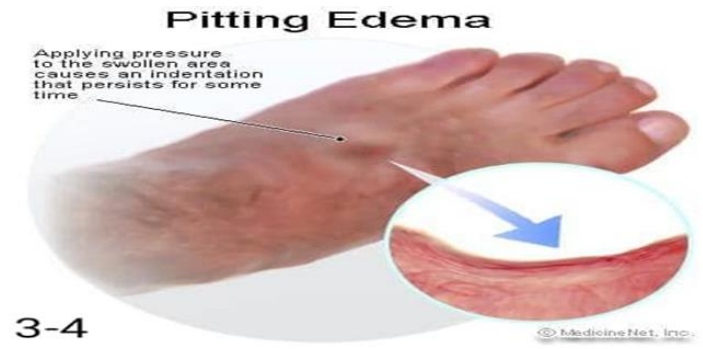
Clinical significance of albumin

Edema: hypoalbuminemia result in fluid retention in the tissue spaces (Figure3-4)

Hypoalbuminemia: low level of albumin is seen in the following conditions :
cirrhosis of liver, malnutrition, nephrotic syndrome, ,burns and Malabsorption .
In cases of burns the fluid inside vessels out of blood and then we loss plasma and its protien (albumin)

Analbuminemia: congenital disorder

Hyperalbuminemia: In conditions of fluid depletion such as
In diarrhea, excessive vomiting, excessive sweating and diuresis
Albumin binds different drugs and strongly affects the pharmacokinetics of these drugs :
For example, sulfonamides can cause the release of unconjugated bilirubin from albumin by competitive binding.
Kernicterus is a type of brain damage that can result from high levels of bilirubin in a baby's blood and it has symptoms of jaundice(figure 3-5)


Pitting Edema
Applying pressure to the swollen area causes an indentation that persists for some time
3-4
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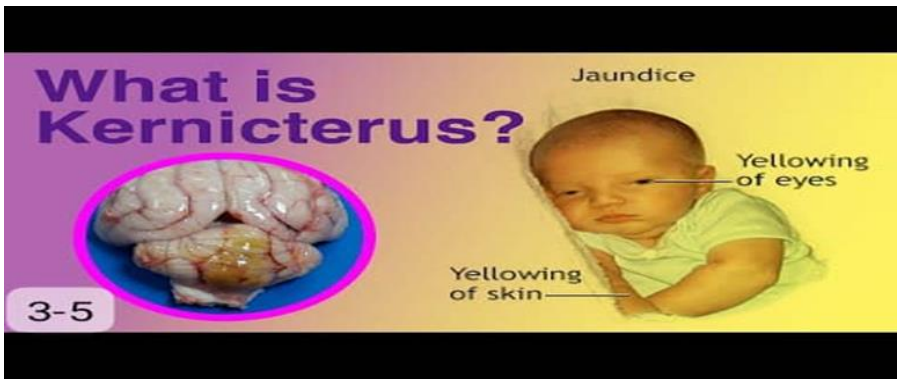
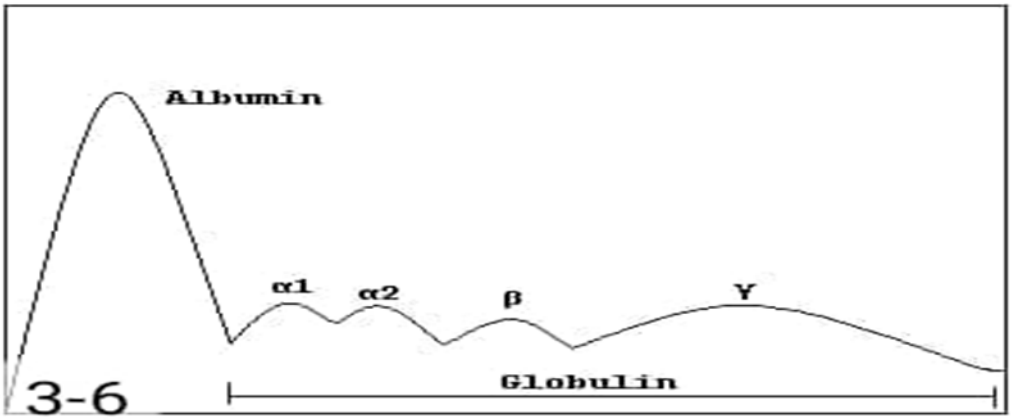


Globulins :

Molecular weight ranges from 90,000 to 13,00,000
By **electrophoresis** globulins can be separated into:
α-globulins
α2-globulins
β-globulins
Y-globulins
(Figure3-6)



Synthesis of Globulins

Alpha , ßeta and gamma globulins are synthesized in the liver.
Y globulins are also synthesized in plasma cells and B-cells of lymphoid tissues (Reticulo- endothelial system)

Synthesis of Y globulins increase in chronic infections, chronic liver diseases, auto-immune diseases, leukemias, lymphomas and various other malignancies

α-Globulins

They are glycoproteins , Based on electrophoretic mobility they are classified into **α1**, and **α_2** globulins

Examples of α1, globulins :
-α 1 antitrypsin
-Orosomucoid (α- acid glycoprotein)
- α-fetoprotein AFP

First fraction of a1 globulins, α-antitrypsin or α, antiprotease : is a single chain protein of 394 amino acids, contains three oligosaccharide chains. Its the major component (>90%) of α_1 globulins
- It is synthesized by hepatocytes and macrophages and it is the principal serine protease inhibitor of human plasma. It inhibits trypsin, elastase, and certain other proteases by forming complexes with them

Clinical consequences of α-antitrypsin deficiency

-**Emphysema** occurs when lack of antitrypsin
Normally antitrypsin protects the lung tissue from proteases (active elastase) that released by macrophages,

α-antitrypsin Forms a complex with protease and inactivates it , in its deficiency, the active elastase destroys the lung tissue by proteolysis

Smoking & Emphysema and relationship between them

A methionine (residue 358) of α-antitrypsin is involved in the α-antitrypsin binding to proteases , the smoking oxidizes this methionine into methionine sulfoxide and thus inactivates α-antitrypsin. α- AT with methionine sulfoxide can not bind to and inactivates the proteases . Emphysema is more commonly associated with heavy smoking.

-a **Cirrhosis** of Liver: Due to the accumulation of a mutant a1- AT which aggregates to form polymers, these polymers cause liver damage (hepatitis) followed by accumulation of collagen resulting in fibrosis (cirrhosis).

The second fraction of a1 globulins , Orosomucoid /α1 acid glycoprotein :
Concentration in plasma is 0.6 - 1.4 g/dl it Carbohydrate content 41%
marker of acute inflammation and acts as a transporter of progesterone Transports carbohydrates to the site of tissue injury .

It Concentration increases in inflammatory diseases, cirrhosis of liver and in malignant conditions while , Concentration it decreases in liver diseases , malnutrition nephrotic syndrome.

The third fraction of a1 globulins(a-fetoprotein (AFP):
-Presence in a high concentration in fetal blood during pregnancy
-normal concentration in healthy adult about 1µg/100ml and its Level increase during pregnancy .
-Clinically considered a tumor marker for the diagnosis of hepatocellular carcinoma or teratoblastomas.

α2-Globulins

-Haptoglobin
-Ceruloplasmin
-α2 macroglobulins

the **first** ftaction of **a2 globulins (Haptoglobin-(Hp))**

-It is a plasma glycoprotein that binds extracorpuscular hemoglobin (Hb) in a tight non-covalent complex (Hb-Hp).

-The amount of Haptoglobin in human plasma ranges from 40 mg- 180 per deciliter , and the main function of Hp is to prevent loss of free hemoglobin into the kidney. This conserves the valuable iron present in hemoglobin, which would otherwise be lost to the body.
-The molecular mass of hemoglobin is approximately 65 kDa , Hb-Hp complex has a molecular mass of approximately 155 kDa.
-Free hemoglobin passes through the glomerulus of the kidney, enters the tubules, and tends to precipitate there in (as can happen after a massive incompatible blood transfusion, when the capacity of haptoglobin to bind hemoglobin is grossly exceeded). However, the Hb-Hp complex is too large to pass through the glomerulus. Thus Hp helps to conserve iron.

-Haptoglobin's Concentration rises in inflammatory conditions and decreases in hemolytic anemias

-Half-life of haptoglobin is approximately 5 days, the half-life of the Hb-Hp complex is about 90 minutes, the complex being rapidly removed from plasma by hepatocytes. Thus, when haptoglobin is bound to hemoglobin, it is cleared from the plasma about 80 times faster than normally.

-The level of haptoglobin falls rapidly in hemolytic anemias.

The **second** fraction of globulins, **Ceruloplasmin**

-Copper containing α2-globulins and
glycoprotein with enzyme It has a blue color because of its high copper content

-Carries 90% of the copper present in plasma

-Each molecule of ceruloplasmin binds six atoms of copper very tightly, so that the copper is not readily exchangeable. copper is needed for the action of many Enzymes such as (ferroxidase, copper oxidase and Histaminase) .

Ceruloplasmin synthesized in liver in the form of apo- ceruloplasmin, when copper atoms get attached it called Ceruloplasmin.
though carries 90% of the copper present in plasma. but it binds copper very tightly, so that the copper is not readily exchangeable.
-Albumin carries the other 10% of the plasma copper but binds the metal less tightly than does ceruloplasmin. albumin thus donates its copper to tissues more readily than ceruloplasmin and appears to be more important than ceruloplasmin in copper transport in the human body.

Clinical Significance of Ceruloplasmin

-Normal level-25-50 mg/dl
-Low levels of ceruloplasmin are found in **Wilson disease** (hepatolenticular degeneration)
-The amount of ceruloplasmin in plasma is also decrease in liver diseases, malnutrition and nephrotic syndrome.

Wilson disease is a rare inherited disorder that mutated of gene that synthesized the ceruloplasmin , Leads to prevent your body from getting rid of extra copper. Too much copper can poison the body. Wilson disease first attacks the liver and causes cirrhosis ,and the central nervous system or both, and can causes parkansonism and motor dysfunction(figure 3-7(

Wilson disease is treated by penicillamine which binds copper (chelation (and leads to excretion of copper in the urine

3-7

The **third** fraction of globulins **(α2- Macroglobulin (AMG**):

-Major component of a2 globulins proteins

-comprises 8-10% of the total plasma protein in humans.

-tetrameric protein with molecular weight of 725,000.

-Synthesized by hepatocytes and macrophages

-Inactivates all the proteases and thus is an important in vivo anticoagulant

-Carrier of many growth factors - its normal serum level-130-300 mg/dl
- α2- Macroglobulin Concentration is markedly increased in nephrotic syndrome

ß Globulins including these fractions : -

1 Transferrin
2 C-reactive protein
2 Haemopexin
3 Complement Clq
3 ß Lipoprotein(LDL)

Transferrin

-It is a glycoprotein (76 kDa) , part of ß fraction.
-It can transport iron in plasma as ferric ions (Fe3+) and protect the body against the toxic effects of free iron,
-Low level of transferrin will result in iron deficiency anemia
-There are receptors (TfR1 and TfR2) on the surfaces of many cells for transferrin.
It's binds to these receptors and is internalized by receptor-mediated endocytosis.
The acid pH inside the lysosome causes the iron to dissociate from the protein.
The dissociated iron leaves the endosome via DMT1 to enter the cytoplasm.
ApoTf is not degraded within the lysosome. Instead, it remains associated with its receptor, returns to the plasma membrane, dissociates from its receptor, reenters the plasma, picks up more iron, and again delivers the iron to needy cells.
Normally, the iron bound to Tiff turns over 10-20 times a day.))(Figure 3-9)

C-reactive protein (ß Globulin)

So named because it reacts with C- polysaccharide of capsule of pneumococci
Molecular weight of 115-140 kD
Synthesized in liver
can stimulate complement activity and macrophages
acute phase protein- concentration rises in inflammatory conditions
Clinically important marker to predict the risk of coronary heart disease

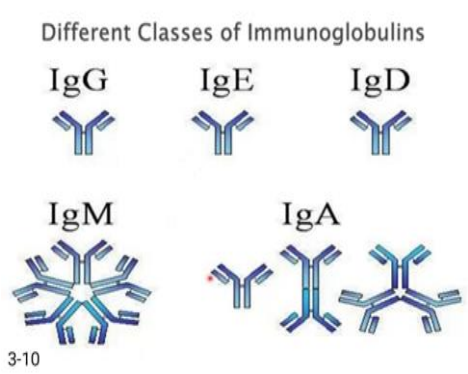
Acute phase proteins

The levels of certain proteins may increase in blood in response inflammatory and neoplastic conditions, these are called Acute phase proteins.

Examples of acute phase protein : -
C-reactive proteins
Ceruloplasmin
Alpha-1 antitrypsin
Alpha 2 macroglobulins
Alpha-1 acid glycoprotein

Antibodies (Immunglobulins) gamma globulins

•Immunoglobulins are a group of proteins (gamma globulins) produced by the body from B lymphocytes and plasma cells in response to presence of foreign bodies (antigens(
There are five types of immunoglobulins IgG, IgA, IgM, IgD, and IgE(figure 3_10)



Major functions of immunoglobulins

Immunoglobulin	Major Functions
IgG	Main antibody in the secondary response. Opsonizes bacteria, Fixes complement, neutralizes bacterial toxins and viruses and crosses the placenta.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membranes. Does not fix complement.
IgM	Produced in the primary response to an antigen. Fixes complement. Does not cross the placenta. Antigen receptor on the surface of B cells.
IgD	Uncertain. Found on the surface of many B cells as well as in serum.
IgE	Mediates immediate hypersensitivity

Fibrinogen
-Also called clotting factor1

-Constitutes 4-6% of total protein

-Large asymmetric molecule

-almparts maximum viscosity to blood Synthesized in liver

-Made up of 6 polypeptide chains,Chains are linked together by S-S linkages

-Amino terminal end is highly negative due to the presence of glutamic acid and the negative charge contributes to its solubility in plasma and prevents aggregation due to electrostatic repulsions between the fibrinogen molecules

Introduction to endocrine system and hormones

The multiple activities of cells, tissues, and organs of the body are coordinated by interplay of several types of chemical messenger systems , but conventionally we say that That the Nervous system and endocrine system constitute the control system in the body .

The term hormone (hormao=to excite) was first used by William M.Bayliss and his brother-in-law Ernest H. Starling, both of London University College, in 1904, who showed that a chemical substance (secretin) from the intestine could stimulate the action of a pancreatic secretion.

The hormone is an organic chemical substance carries a signal , hormones can be :

1 **endocrine hormones** which are released by glands or specialized cells into circulating blood and influence the function of target cells at another location in the body

2. **Neuroendocrine hormones** which are secreted by neurons into circulating blood and influence the function of target cells at another location in the body

3.**Paracrine hormones** which are secreted by cells into extracellular fluid and affect neighboring target cells of a different types

4.**Autocrine hormones** which are secreted by cells into extracellular fluid and affect the function of the same cells

Important of hormones:

-Regulation of metabolism

-Regulation of growth and development

-Regulation of water and electrolyte balance

-Other functions

Hormones can be classified according to the chemical structure into

1.**Proteins** and **polypeptides** such as insulin and glucagon

2.**Steroids** such sex hormones and cortisol

3 **Amine hormones** "derivatives of tyrosine amino acid" which are thyroid and adrenal medullary hormones

(Figure 4-1)

Amino acid hormones	Steroid hormones	Polypeptide hormones
thyroxine triiodothyronine adrenaline noradrenaline melatonin	cortisol oestradiol aldosterone androgens progesterone testosterone active form of vitamin D_3	antidiuretic hormone (ADH) oxytocin insulin pituitary trophic hormones calcitonin parathyroid hormone prolactin (PRL) growth hormone (GH) lipotrophin (LPH)

figure 4-1

Hormones also classified according to the solubility properties into two groups

Group I "water insoluble"

Group II "water insoluble"

Hormones can be classified into two main groups:

	Group I	Group II
Solubility	Lipophilic	Hydrophilic
Chemically	Steroids, Thyroid hormones	Amino acids derivatives
Plasma Transport protein	Yes	No
Plasma half life	Long (hours to days)	Short (minutes)
Receptor	Intracellular (cytosolic , nuclear)	Cell surface receptor
Mediator	Receptor-hormone complex	Second messengers: cAMP, Ca2+, phospho-inositols, tyrosine kinase

figure 42

Mechanism of action of hormones

The 1st step of a hormone's action is to bind to specific receptors at the target cell "cell that lack receptors for hormone will not respond . The binding of hormone to it's receptor this activates the receptor, and the activated receptor initiates intracellular events which can cause :

-Changes in membrane permeability or electrical state
-Synthesis of proteins, such as enzymes
-Activation or inactivation of enzymes
-Stimulation of mitosis
-Promotion of secretory activity

hormone receptors

Hormone receptors are large proteins , and each cell that is to be stimulated usually has some 2000 to 100,000 receptors , also each receptor is usually highly specific for a single hormone .the receptor can be located at :

-Cell surface

-Cytoplasm

-nucleus

-Even in mitochondria

Note that the receptor of a hormone may located **at more than one place** on a cell .

Types of receptors

1**. Ion channel-linked receptors** : open or close the ion channels which alter the ions movement and electrical potential of the target cell , but the usage of this type of receptors by hormones is uncommon , usually hormones open the ion channels indirectly .

2. **G Protein-linked receptors**

Many hormones activate receptors that indirectly regulate the activity of target proteins (e.g., enzymes or ion channels) by coupling with groups of cell membrane proteins called heterotrimeric guanosine triphosphate (GTP)-binding proteins (G proteins) (Figure 4-3). Of more than 1000 known G protein-coupled receptors, all have seven trans-membrane segments that loop in and out of the cell membrane. Some parts of the receptor that protrude into the cell cytoplasm (especially the cytoplasmic tail of the receptor) are coupled to G proteins that include three (ie., trimeric) parts--the α, B, and y subunits. When the

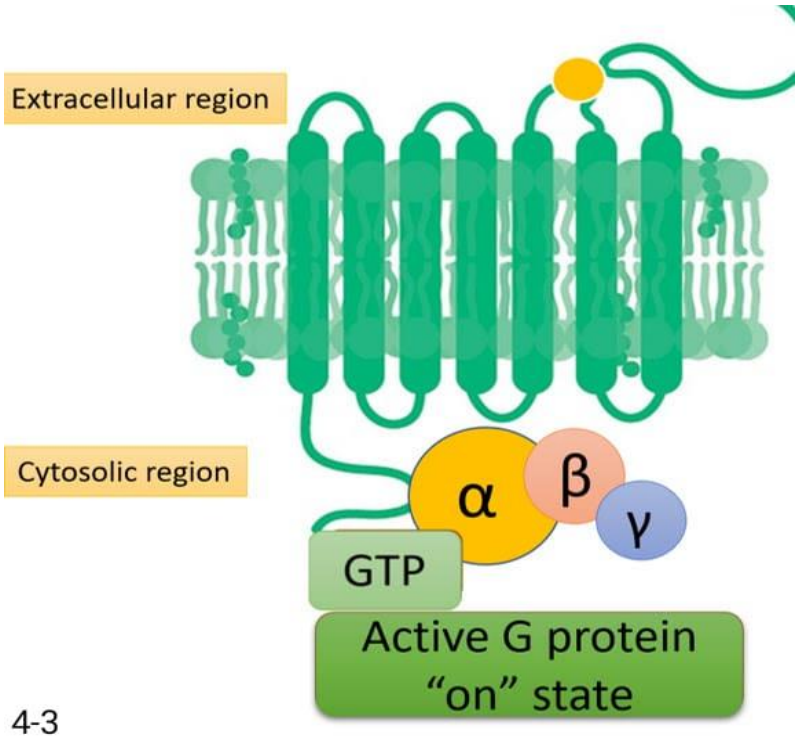
ligand (hormone) binds to the extracellular part of the receptor, a conformational change occurs in the receptor that activates the G proteins and induces intra- cellular signals that either (1) open or close cell membrane ion channels, (2) change the activity of an enzyme in the cytoplasm of the cell, or (3) activate gene transcription .

The trimeric G proteins are named for their ability to bind guanosine nucleotides. In their inactive state, the α, B, and y subunits of G proteins form a complex that binds guanosine diphosphate (GDP) on the a subunit. When the receptor is activated, it undergoes a conformational change that causes the GDP-bound trimeric G protein to associate with the cytoplasmic part of the receptor and to exchange GDP for GTP. Displacement of GDP by GTP causes the "a" subunit to dissociate from the trimeric complex and to associate with other intracellular signaling proteins; these proteins, in turn, alter the activity of ion channels or intracellular enzymes such as adenylyl cyclase or phospholipase C, which alter cell function.

The signaling event is terminated when the hormone is removed and the a" subunit inactivates itself by convert- bound GTP to GDP; then the a" subunit once again combines with the ß and y subunits to form an inactive, membrane-bound trimeric G protein.

Some hormones are coupled to inhibitory G proteins "Gi" whereas others are are coupled to stimulatory G proteins "Gs" . Thus depending on the coupling of a hormone receptor to an inhibitory or stimulatory G protein , a hormone can either increase or decrease the activity of intracellular proteins .

The Gs is stimulates Adenylyl cyclase , while Gi inhibit it . There is also a "Gq" which stimulates Phospholipase C .



4-3

3. **Enzyme-linked receptors "such as tyrosine kinase "**

Some receptors, when activated, function directly as enzymes or are closely associated with enzymes that they activate. These enzyme-linked receptors are proteins that pass through the membrane only once, in contrast to the seven- trans-membrane G protein-coupled receptors. Enzyme - linked receptors have their hormone-binding site on the outside of the cell membrane and their catalytic or enzyme-binding site on the inside. When the hormone binds to the extracellular part of the receptor, an enzyme immediately inside the cell membrane is activated . Although many enzyme-linked receptors have intrinsic enzyme activity, others rely on enzymes that are closely associated with the receptor to produce changes in cell function. Many hormone receptors found to have an intrinsic tyrosine kinase activity activated by binding of their ligands such as insulin hormone and Epidermal growth factor .

Mode of action of hormones

The hormones alter the cellular functions by change:

A. **Activity** of specific proteins

B. **Amount** of specific proteins

Because group I hormones are lipid soluble "=water insoluble" they defuse across the cell membrane easily , and bind to intracellular receptors "either cytoplasmic or nuclear receptors" and form a hormone receptor complex which can bind to the DNA at specific regions called hormone receptor elements , this will affect the transcription " either by increasing or decreasing" and subsequently the synthesis "amount" of specific proteins. Figure 4-4

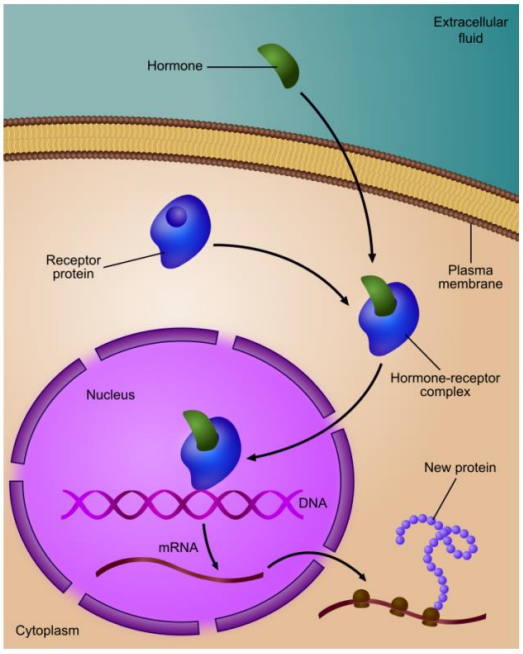


figure 4-4

This mode of action is slow " minutes, hours, or even days required for cause a significant response" .

Note that some group I hormones have a cell membrane receptors beside their intracellular receptors.

Many different tissues can have the same intracellular hormone receptor, but the genes that the receptor regulate are different in various tissues. An intracellular receptor can regulate gene expression only if appropriate combination of gene regulatory proteins are present, and many regulatory proteins are tissue-specific . Thus, the response of different tissues to a hormone determined not only by specificity of receptors but also by the type of tissue " transcriptionally active DNA in the tissue" .

Mechanism of action of group II

Because hormones of group II are lipid insoluble they can't diffuse across the cell membrane. They bind to cell surface receptors . The hormone (first messenger) binds to a specific receptors located In the cell membrane to activate a second messenger which Is located In the cytosol.

The second messenger may be one of the following molecules

cyclic AMP (cAMP)

cyclic GMP

membrane phospholipids

Ca^{++}

Note : the specific action that occurs in response to increase or decrease the 2nd messenger in each type of target cell is depends on the nature of intracellular machinery "the set of enzymes and proteins in these cell " , for example both thyroid stimulating hormone and antidiuretic hormone are use the same 2nd messenger "cAMP" and because the follicular cells "in thyroid" and tubular cells "kidneys" have different set of enzymes and proteins they respond by different responses to the same signal "increase cAMP level" i.e TSH stimulates the synthesis and release of Thyroid hormones, while the ADH increases the permeability of collecting tubules to water .

cAMP

cAMP is formed from ATP in reaction catalyzed by Adenylyl cyclase. cAMP was the first intracellular second messenger signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP .

cAMP stimulates an enzyme called protein kinase A " cAMP-dependant protein kinase". This enzyme is composed of 4 subunits: 2 regulators (R) and 2 catalytic (C). The whole protein kinase Is Inactive, but binding of cAMP with the enzyme dissociates R from C and activating it. The protein kinase A then phosphorylates many substrates , this phosphorylation either cause activation or inactivation , and thus cause a change in the cell function.(Figure 4-5)

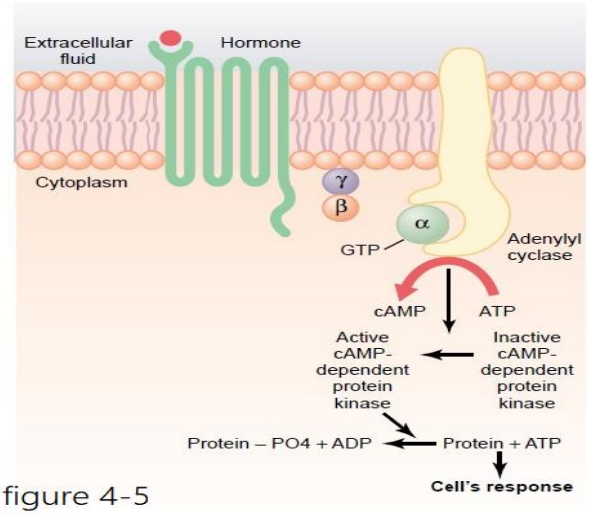


figure 4-5

cGMP

cGMP is formed from GTP in a reaction catalyzed by Guanylyl cyclase, which exist in soluble and membrane-bound forms .

The atriopeptines , a family of peptides produced in the atrial tissues of heart and cause natriuresis , diuresis and vasodilation are bind to an activate the membrane bound form of Guanylyl cyclase. This will result in increase the. cGMP "up to 50 fold" . cGMP activates the cGMP-dependant protein kinase which phosphorylates many substrates and their activities and thus the cell function .(Figure 4-6)

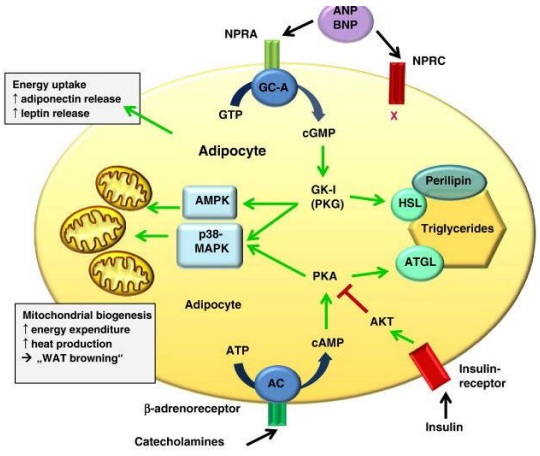


figure 4-6

Cell membrane phospholipids

Some hormones activate transmembrane receptors that activate the enzyme Phospholipase C attached to the inside projections of receptors (figure 4-7). This enzyme catalyzes the breakdown of some phospholipids in the cell membrane, especially phosphtidylinositol biphosphate (PIP2) into two different second messengers: inositol triphosphate (IP3) and diacylglycerol (DAG). The IP3 mobilizes calcium ions from mitochondria and endoplasmic reticulum, and calcium ions then have their own second messenger effects .

DAG, the other lipid second messenger, activates enzyme protein kinase C , which then phosphorylates many substrates leading to cell's response (figure 4-7) .in addition to these effects, the lipid portion of DAG is arachidonic Acid which is the precursor for prostaglandins and other local hormones that cause multiple effects in tissues throughout the body

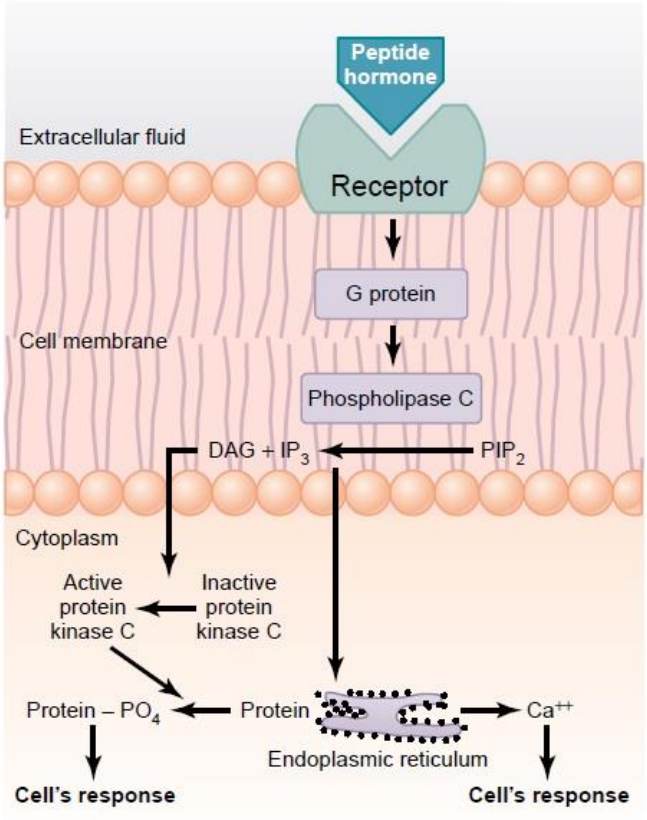


figure 4-7

Calcium ions

Ionized calcium bind to and activates the calcium-dependant regulatory protein know as calmodulin (figure 4-8) , Ca^{++}-Calmodulin complex then activates many proteins result in cell response

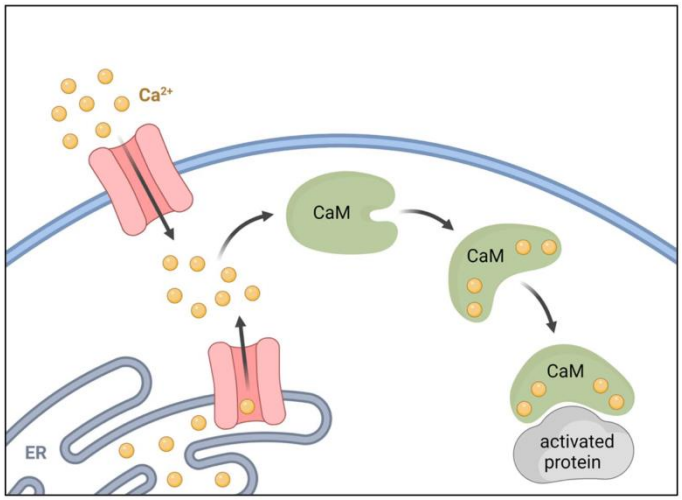


figure 4-8

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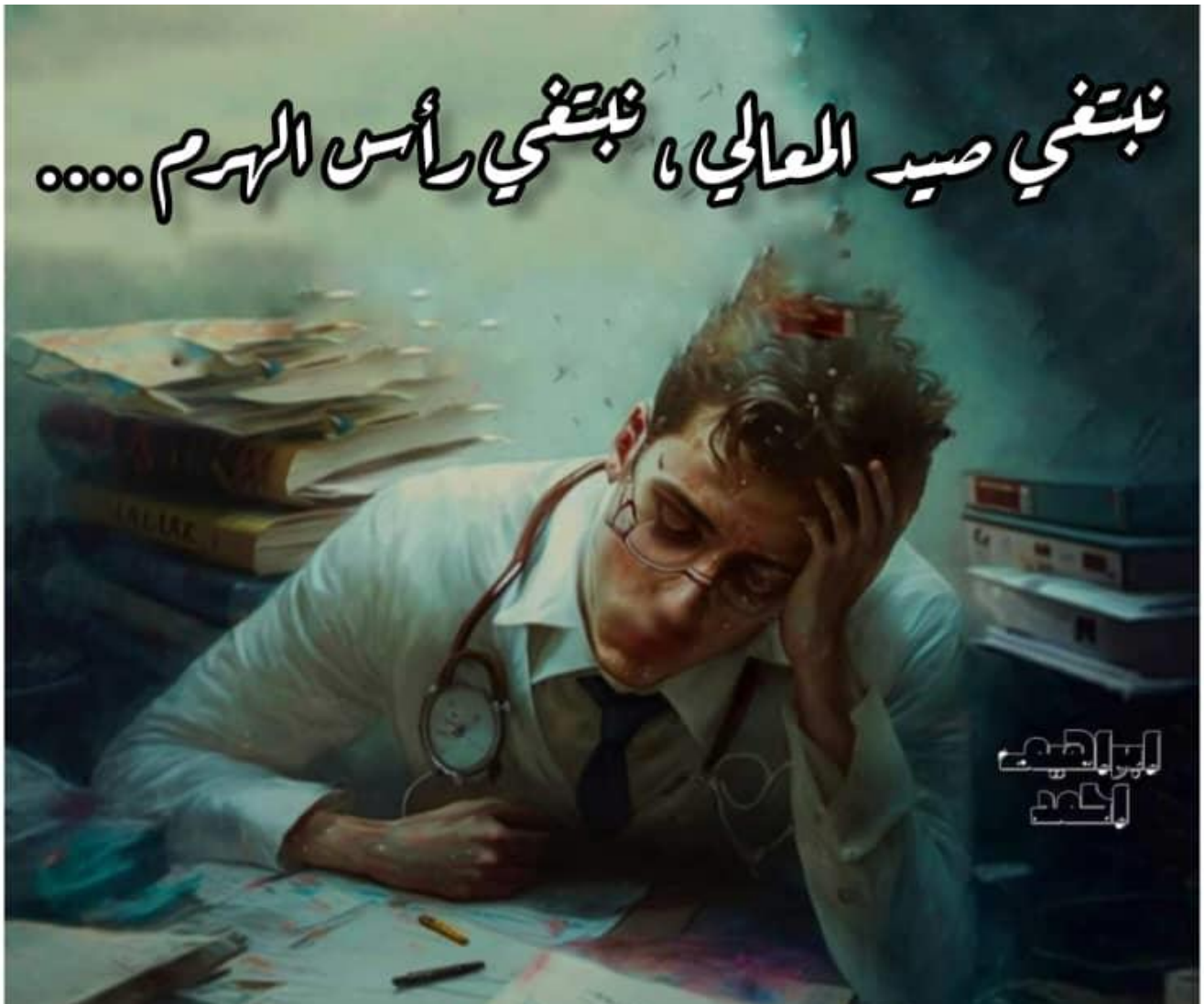
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ابراهيم أحمد